YEASTS

A news letter for persons interested in yeasts.

December 1950 Volume I, number 1

Editor, 1950-51: Leslie R. Hedrick, Illinois Institute of Technology, Chicago
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This is the first issue of a communication which we hope will continue to be published and distributed for many years. Although the contributors for this number all reside in the United States, it is contemplated that soon contributions and distributions will be international in scope. The purpose of YEASTS is to provide a channel of communication for persons interested in research and teaching with yeasts. The suggestion has been made that the editorship should be rotated annually among persons in different regions of the United States. Dr. Mrak of the University of California has agreed to serve as associate editor this year to solicit information from various persons in the western states. Dr. Etchells is associate editor for the eastern states. It is evident that if such an organization is to develop on a democratic basis, some mechanism must be provided for the interested persons to vote on an organizational constitution.

It has already been suggested that perhaps we could ask the program committee of the Society of American Bacteriologists, or the Mycological Society, to group all the yeast papers in one session. Desires have been expressed for a meeting of the group at either the S.A.B. or the Mycological Society annual meetings.

In order to obtain an expression of opinion from persons on our mailing list, an opportunity is provided for indicating the desires of each person on the special sheet enclosed.

We plan to publish at least one more issue this year, probably in March or April. If we receive enough material from interested people, a third number might be prepared for distribution in early June. Any suggestions or comments will be greatly welcomed by myself or the associate editors.

Leslie R. Hedrick

Leslie R. Hedrick

December 6, 1950

The Yeast Newsletter
Volume 50

This issue marks the 50th anniversary of the Yeast Newsletter. The frontispiece is from the original issue put out by Leslie Hedrick (Illinois Institute of Technology) in 1950. The Editorship was intended to rotate annually, but after the one year tenure of Emil Mrak (U.C. Berkeley), the job settled for the next 34 years upon Herman Phaff (U.C. Davis). The first issue was mailed to 46 researchers in the United States, but the readership rapidly expanded to several hundreds worldwide. Currently the Yeast Newsletter is mailed to readers in nearly 50 countries. The original optional subscription fee of $0.25 has followed inflation to the current level of $8.00 (increases are not anticipated in the near future). Coincidentally, this number is also the longest so far, due in good part to contributions made through the Food and Beverages Yeast Network, under the coordination of Patrizia Romano.

Several readers have suggested that the time has come to move the Yeast Newsletter to the Internet. I am exploring the possibility of using the World Wide Web to inform potential readers of the existence of our publication, and possibly to facilitate communications among our readers. However, based on my own use of Internet resources compared to the time I spend reading "hard copy" books, journals, or magazines, I am convinced that a printed copy mailed to all our readers remains, at this time, the vehicle of choice to exchange information on our research activities, to report on past meetings, to plan for forthcoming conferences, and to help yeast researchers keep in touch with one another.

I thank our readers who, over the years, have taken the time to send regular contributions.

M. A. Lachance
Editor

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Yeast

A Newsletter for Persons Interested in Yeast

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Editorials

In Memoriam - Prof. Aksel Stenderup

I am saddened by the recent news of the passing of Aksel Stenderup, Professor emeritus, Institute of Medical Microbiology, University of Aarhus, Denmark, on February 14, 2001. He was 81 yeas old. I first met Dr. Stenderup in Hungary in 1977, where he impressed me with his wry sense of humour. I later interacted with him in his capacity as member of Executive Committee of IUMS. He will be remembered by many for his work on opportunistic pathogenic yeasts.

In Memoriam - Prof. Helen R. Buckley

It is with much regret that I was informed of the passing of Helen Buckley, Professor of Microbiology and Immunology at Temple University Hospital and School of Medicine, Philadelphia, on February 28, 2001, at age 65. In addition to her contributions in the pathogenesis of *Candida albicans*, Dr. Buckley was an award-winning teacher. She was to receive, later this year, the 2001 William A. Hinton Award of ASM in recognition for her contributions to research training of minority students. She served as past President of the Medical Mycological Society of the Americas. She will be missed by all who had the pleasure of knowing her.

FEMS Yeast Research

The first issue of *FEMS Yeast Research* appeared in April, although papers have been available in electronic form practically from the moment they were accepted. The second issue is in the press. Chief Editor Lex Scheffers defined for the journal the threefold objective of providing a communication vehicle for all aspects of yeast research, making quality the sole criterion for acceptance of papers, and assuring rapid publication with a minimum of bureaucratic hindrance. He appears to have succeeded. The first issue contains a mix of papers on the genetics, ecology, physiology, systematics, and applied biology of yeasts. As for quality, readers can judge for themselves that the editorial board has adhered to high standards. I can certainly attest to the efficiency of the editorial office and their quick response at all stages of the publication process. Of course, the establishment of *FEMS Yeast Research* as a first rate journal will be predicated on the continued support of authors who will submit their best work. Therefore, I again invite readers of the Yeast Newsletter to make *FEMS Yeast Research* their first choice for the submission of their most significant articles on all aspects of yeast biology. The contents of both issues and notes for authors can be found on the web site, where abstracts and full texts are freely accessible: http://www.fems-microbiology.org/fems/publications/journals/htm

Payment by Credit Card

Our first experience with credit cards as a means of payment has been mostly positive. It is now possible for readers in some parts of the world to avoid paying very high bank charges for the issuance of cheques. However, the management of credit card invoices has caused a considerable increase in the amount of work required to balance our accounts and a sharp increase in administrative costs. I must therefore ask our readers who wish to pay by credit card (1) to do so only for advance payments of $40.00 or more, (2) to make their payment as soon as possible after receipt of the annual invoice, in January, and (3) to insure that their card expiry date is not within the next four months.

M.A. Lachance
Editor

I. Centraalbureau voor Schimmelcultures, Yeast Identification Service, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. Communicated by M.Th.Smith <smith@cbs.knaw.nl>.

Recent acquisitions. For recent acquisitions, please consult the catalogue on the www at www.cbs.knaw.nl/www/cbshome.html.
CBS website. Online searches and similarity identifications of yeasts species are now possible on the CBS web site: www2.cbs.knaw.nl/yeast/webc.asp. All available data (including administrative, morphological and physiological data, as well as sequences, gels, images, bibliography, taxonomy …) related to all CBS strains (~4500) and to 719 currently recognized species are recorded.

The following articles have appeared or are in press.

1. De Rijk, P., Robbrecht, E., Hoog, G.S. de, Caers, A., Van de Peer, Y. & De Wachter, R. 1999. Database on the structure of large subunit ribosomal RNA. Nucl. Acids Res. **27**:174-178.

2. Van de Peer, Y., Robbrecht, E., Hoog, G.S. de, Caers, A., De Rijk, P. & De Wachter, R. 1999. Database on the structure of small subunit ribosomal RNA. Nucl. Acids Res. **27**:179-183.

3. Aanen, D.K., Kuyper, Th.W., Boekhout, T. & Hoekstra, R.F. 2000. Phylogenetic relationships in the genus *Hebeloma* based on ITS 1 and 2 sequences, with special emphasis on the *Hebeloma crustuliniforme* complex. Mycologia **92**:269-281.

4. Begerow, D., Bauer, R. & Boekhout, T. 2000. Phylogenetic placements of ustilaginomycetous anamorphs as dededuced from LSU rDNA sequence. Mycol. Res. **104**:53-60.

5. Boekhout, T. 2000. De soortenrijkdom van gistten. Cahiers Biowetenschappen en maatschappij **20**:11-17.

6. Boekhout, T., Fell, J.W., Fonseca, A., Prillinger, H.J., Lopandic, K. & Roeijmans, H.J. 2000. The basidiomycetous yeast *Rhodotorula yarrowii* comb. nov. Antonie van Leeuwenhoek **77**:355-358.

7. Fell, J.W., Boekhout, T., Fonseca, A. & Sampaio, J.P. 2000. Basidiomycetous yeasts. In McLaughlin, D.J., McLaughlin, E.G. & Lemke, P.A. (eds): The Mycota VII part B. Springer, Berlin, pp. 1-35.

8. Fell, J.W., Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman, A. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int. J. Syst. Evol. Biol. **50**:1351-1371.

9. Gunde-Cimerman, N., Zalar, P. & de Hoog, G.S. 2000. Hypersaline water in salterns - natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecol. **32**:235-240.

10. Hoog, G.S. de, Matos, T., Rainer, J., Peltroche-Llacsahuanga, H., Haase, G., Vicente, V., Attili Angelis, D., Gerrits van den Ende, A.H.G., Pizzirani- Kleiner, A.A., Fernandez-Zeppenfeldt, G., Richard-Yegres, N., Yegres, F. & Queiroz-Telles, F. 2000. Black fungi: clinical and pathogenic approaches. Med. Mycol., Suppl. **1**:243-250.

11. Hoog, G.S. de, Mayser, P., Haase, G., Horré, R. & Horrevorts, A.M. 2000. A new species of *Phialophora* causing superficial infections in humans. Mycoses **43**:409-416.

12. Hoog, G.S. de, Guarro, J., Gené, J. & Figueras, M.J. 2000. Atlas of Clinical Fungi, 2nd ed. Utrecht/Reus, 1126 pp.

13. Meletiadis, J., Meis, J.F.G.M., Hoog, G.S. de & Verweij, P.E. 2000. *In vitro* susceptibilities of 11 clinical isolates of *Exophiala* species to six antifungal drugs. Mycoses **43**:309-312.

14. Middelhoven, W.J., Guého, E. & Hoog, G.S. de 2000. Phylogenetic position and physiology of *Cerinosterus cyanescens*. Antonie van Leeuwenhoek **77**:313-320.

15 Müller, W.H., Stalpers, J.A. Van Aelst, J.A. De Jong, M.D.M. Van Der Krift, Th.P. & Boekhout, T. 2000. The taxonomic position of *Asterodon*, *Asterostroma* and *Coltricia* inferred from the septal pore cap ultrastructure. Mycol. Res. 104:

16. Müller, W.H., Humbel, B.M., Van Aelst, A.C., Van der Krift, T.P., Verkleij, A.J., and Boekhout, T. 2000. Field- emission scanning electron microscopy of the internal cellular organization of fungi. Scanning **22**:295-303.

17. Müller, W.H., Koster, A.J., Humbel, B.M., Ziese, U., Verkleij, A.J., van Aelst, A.C., van der Krift, T.P., Montijn, R.C. & Boekhout, T. 2000. Automated electron tomography of the septal pore cap in *Rhizoctonia solani*. J. Struct. Biol. **131**:10-18.

18. Müller, W.H., van Aelst, A.C., Humbel, B.M., van der Krift, T.P. & Boekhout, T. 2000. Field-emission scanning electron microscopy of the internal cellular organization of fungi. Scanning **22**:295-303.

19. Smith, M.Th., Yarrow, D. & Robert, V. 2000. Chapter 1. Identification of the common food- and airborne fungi. Yeasts. In: (eds. Samson et al.) Introduction to food- and airborne fungi pp. 270-278. Centraalbureau voor Schimmelcultures, Utrecht.

20. Avis, T.J., Caron, S.J., Boekhout, T., Hamelin, R.C. & Bélanger, R.R. 2001. Molecular and physiological analysis of the powdery mildew antagonist *Pseudozyma flocculosa* and related fungi. Phytopathology **91**:249- 254.

21. Boekhout, T. & Guého, E. 2001. Basidiomycetous yeasts. In: Pathogenic fungi of humans and animals (Howard, D., ed.), Dekker, New York (in press).

22. Boekhout, T., Robert, V., Yarrow, D., Smith. M.Th., Kurtzman, C.P., Fell, J.W., Roberts, I., Gijswit, G., Boer, F. 2001. Yeast species of the world - a CD- ROM, Expertise Center Taxonomic Identification (ETI), University of Amsterdam.

23. Boekhout, T., Theelen, B., Diaz, M., Fell, J.W., Hop, W.C.J., Abeln, E.C.A., Dromer, F. & Meyer, W. 2001. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. Microbiology **147**:891-907.

24. Diaz, M.R., Boekhout, T., Theelen, B. & Fell, J.W. 2001. Molecular sequence analyses of the intergenic spacer (IGS) associated with rDNA of the two varieties of the pathogenic yeast, *Cryptococcus neoformans*. Syst. Appl. Microbiol. **23**:535-545.

25. Gildemacher, P., Heijne, B., Boekhout, T. & Hoekstra, E. 2001. Fungiciden verminderen verruwwing, maar niet voldoende. Fruitteelt **2**:10-11.

26. Kerrigan,J.,Smith, M.Th., Rogers, J.D. & Poot, G.A. 2001. *Ascobotryozyma americana* gen. nov. et sp. nov. and its anamorph *Botryozyma americana*, an unusual yeast from the surface of nematodes. Antonie van Leeuwenhoek **79**:7-16.

27. Naumova, E.S. Smith, M.Th., Boekhout, T., Hoog, G.S. de & Naumov, G.I. 2001. Molecular differentiation of sibling species in the *Galactomyces geotrichum* complex. Antonie van Leeuwenhoek (accepted).

28. Petter, R., Kang, B.S., Boekhout, T., Davis, B.J. & Kwon- Chung, K.J. 2001. A survey of heterobasidiomycetous yeasts for the presence of the gene homologues to virulence factors of *Filobasidiella neoformans*, CNLAC1 and CAP59. Microbiology (in press).

29. Vincent Robert, Bonjean, B., Karutz, M., Paschold, H., Peeters, W., and Marcel G. Wubbolts, M.G. *Candida bituminiphila*, a new anamorphic species of yeast. Int. J. Syst. Evol. Microbiol. (accepted).

30. Theelen, B., Silvestri, M., Guého, E., van Belkum, A. & Boekhout, T. 2001. Identification and typing of *Malassezia* yeasts using amplified fragment length polymorphism (AFLPTm) and denaturing gradient gel electrophoresis (DGGE). FEMS Yeast Research **1**: (in press).

The following articles have been submitted.

31. Cadez, N., Raspor, P., de Cock, A.W.A.M., Boekhout, T. & Smith, M.Th. Molecular identification and genetic diversity within species of the genera *Hanseniaspora* and *Kloeckera*.

32. Göttlich, E., Lubbe, W. Van der, Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., Flemming, H.-C. & Hoog, G.S. de. 2001. The predominant fungal flora in ground water-derived public drinking water.

33. Matos, T., Hoog, G.S. de, Boer, A.G. de, Crom, I. & Haase, G. 2001. High prevalence of the neurotropic black yeast *Exophiala dermatitidis* in bathing facilities.

34. Peltroche-Llacsahuanga, H., Schnitzler, N., Jentsch, S., Platz, A., Hoog, G.S. de, Schweizer, K. & Haase, G. 2000. Comparison of phagocytosis, evoked oxidative burst, and killing of black yeasts analyzing species with different pathogenic potential.

II. Japan Collection of Microorganisms, RIKEN, Wako, Saitama 351-0198, Japan. Communicated by M. Hamamoto <hamamoto@jcm.riken.go.jp>.

For recent acquisitions, please consult the catalogue on the JCM home page at www.jcm.riken.go.jp/.

The following articles have appeared, or are in press.

1. Sugita, T., Takashima, M., Ikeda, R., Nakase, T. and Shinoda, T. 2000. Phylogenetic and taxonomic heterogeneity of *Cryptococcus humicolus* by analysis of the sequences of the internal transcribed spacer regions and 18S rDNA, and the phylogenetic relationships of *C. humicolus*, *C. curvatus*, and the genus *Trichosporon.* Microbiol. Immunol. **44:**455-461.

2. Sugita, T., Takashima, M., Ikeda, R., Nakase, T. and Shinoda, T. 2000. Intraspecies diversity of *Cryptococcus laurentii* as revealed by sequences of internal transcribed spacer regions and 28S rRNA gene and the taxonomic position of *C. laurentii* clinical isolates. J. Clin. Microbiol. **38:**1468-1471.

3. Dao Thi Luong, Takashima, M., Pham Van Ty, Nguyen Lan Dung and Nakase, T. 2000. Four new species of *Kockovaella* isolated from plant leaves collected in Vietnam. J. Gen. Appl. Microbiol. **46:**297-310.

4. Bai, F.-Y., Takashima, M., Hamamoto, M. and Nakase, T. 2001. *Sporobolomyces yunnanensis* sp. nov., a Q-10(H_2)-containing yeast species with a close phylogenetic relationship to *Erythrobasidium hasegawianum*. Int. J. Syst. Evol. Microbio. **51:**231-235.

5. Sugita, T., Takashima, M., Ikeda, R., Nakase, T. and Shinoda, T. 2001. Intraspecific diversity of *Cryptococcus albidus* isolated from humans as revealed by sequences of the internal transcribed spacer regions. Microbiol. Immunol. **45:**291-297.

6. Fungsin, B., Hamamoto, M., Arunpairojana, V., Sukhumavasi, J., Atthasampunna, P. and Nakase, T. 2001. *Bensingtonia thailandica* sp. nov., a novel basidiomycetous yeast species isolated from plant leaves in Thailand. Int. J. Syst. Evol. Microbiol. **51:**1209-1213.

7. Sugita, T., Takashima, M., Nakase, T., Ichikawa, T., Ikeda, R. and Shinoda, T. 2001. Two new yeasts, *Trichosporon debeurmannianum* sp. nov. and *Trichosporon dermatis* sp. nov., transferred from the *Cryptococcus humicola* complex. Int. J. Syst. Evol. Microbiol. **51:**1221- 1228.

8. Nagahama, T., Hamamoto, M., Nakase, T., Takami , H. and Horikoshi, K. Distribution and identification of red yeasts in deep-sea environments around the northwest Pacific Ocean. Antonie van Leeuwenhoek (in press).

9. Takashima, M. and Nakase, T. *Tilletiopsis derxii*, *Tilletiopsis oryzicola* and *Tilletiopsis penniseti*, three new species of the ustilagionomycetous anamorphic genus *Tilletiopsis* isolated from leaves in Thailand. Antonie van Leeuwenhoek (in press).

III. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.

The following are publications for 2001 or in press.

1. Naumov G.I., Naumova E.S., Aigle M., Masneuf I., Belarbi A. 2001. Genetic reidentification of the pectinolytic yeast strain SCPP as a *Saccharomyces bayanus* var. *uvarum.* Appl. Microbiol. Bitechnol. **55**:108-111.

2. Naumova E.S., Tokareva N.G., Bab'eva I.P., Naumov G.I. 2001. Molecular genetic analysis of the yeast *Komagataea* (*Williopsis*) *pratensis* strains isolated from the Caucasian and Tien Shan soils. Microbiology (Engl. Transl.) **70**(2):200-205.

3. Kondratieva V.I., Naumov G.I. 2001. Phenomenon of ascospore lethality (spore killing) in hybrids of *Schizosaccharomyces pombe*. Dokl. Biol. Sciences. (in press).

4. Naumov G.I., Nguyen H.-V., Naumova E.S., Michel A., Aigle M., Gaillardin C. 2001. Genetic identification of *Saccharomyces bayanus* var. *uvarum*, a cider-fermenting yeast. Int. J. Food Microbiol**.** **65**:163-171.

Twenty-one *Saccharomyces* strains isolated from a cider process were analysed in terms of karyotypes, Y' *S. cerevisiae* sequence occurrence, rDNA structure and cross-fertility with species tester strains. A strong predominance of *S. bayanus* var. *uvarum* was found (18 strains *vs.* three *S. cerevisiae*). Among the *S. bayanus* var. *uvarum*, only three strains proved to contain species-specific Y' *S. cerevisiae* sequences.

5. Naumova E.S., Tokareva N.G., Naumov G.I. 2001. *Williopsis saturnus* and *Williopsis beijerinckii* are recognized as distinct taxa by means of polymerase chain reaction with nonspecific primers. Microbiology (Engl. Transl.) **70**(3):355-359.

Fifteen strains of *Williopsis* sensu stricto, analyzed by the polymerase chain reaction with the universal primer N21, were clearly separated into two groups corresponding to the species *W. saturnus* (Klöcker) Zender and *W. beijerinckii* (van der Walt) Naumov et Vustin. The results obtained are in good agreement with the data of genetic hybridization analysis and izosyme electrophoresis, and did not confirm the conspecificity of *W. saturnus* and *W. beijerinckii*.

6. Tokareva N.G., Naumova E.S., Bab'eva I.P., Naumov G.I. 2001. Identification of strains of *Zygowilliopsis californica* having different origin by polymerase chain reaction with universal primers. Microbiology (Engl. Transl.) **70**(4) (in press).

Twenty seven museum strains of different origin have been reidentified by molecular methods. UP-PCR and dot-hybridization showed that only 22 strains belonged to the biological species *Zygowilliopsis californica* (Lodder) Kudriavzev. Four strains are reindentified as *Williopsis suaveolens* (Klцcker) Naumov et al. Universal primers L45 and N21 are recommended for identification of *Z. californica* strains.

7. Naumova E.S., Smith M. Th., Boekhout T., de Hoog G.S., Naumov G.I. 2001. Molecular differentiation of sibling species in the *Galactomyces geotrichum* complex. Antonie van Leeuwenhoek (in press).

PCR-analysis, multilocus enzyme electrophoresis and molecular karyotyping were used to characterize 52 strains belonging to the genus *Galactomyces*. The data obtained revealed that a PCR method employing the universal primer N21 and microsatellite primer $(CAC)_5$ is appropriate for the distinction of four *Ga. geotrichum* sibling species, *Ga. citri-aurantii* and *Ga. reessii*. The better separation was achieved with the UP primer N21; each species displayed a specific pattern with very low intraspecific variation. We propose to use the primer N21 for differentiation of six taxa composing the genus *Galactomyces.* Multilocus enzyme electrophoresis revealed genetic homogeneity of each sibling species within the *Ga. geotrichum* complex. On the other hand, the four sibling species, having from 41 to 59% of nDNA homology and similar phenotypic characteristics, are clearly distinguished based on their electrophoretic profiles using two enzymes: mannose-6-phosphate isomerase (MPI) and phosphoglucomutase (PGM). Despite the same number of chromosomal bands, different karyotype patterns were found in

Ga. geotrichum sensu stricto and its two sibling species A and B. Within each sibling species, chromosome length polymorphism was observed, in particular for small bands, allowing discrimination to the strain level.

8. Naumov G.I., Naumova E.S., Schnürer J. 2001. Genetic characterization of the nonconventional yeast *Hansenula anomala*. (to be published).

We described genetic, molecular and taxonomic characteristics of the yeast *Hansenula anomala*. Pulsed-field gel electrophoresis of chromosomal DNAs from 19 *H. anomala* strains and related species indicated that *H. anomala* had clearly different karyotype. Chromosome length polymorphism of the *H. anomala* strains was independent of their geographic origin and source of isolation. The strains were classified into four groups of similar karyotypes and one strain showed unique profile. The sizes of chromosomes ranged from 850 to 3500 kb in different strains. The haploid chromosome number of *H. anomala* is at least nine. We have found RAPD primers discriminating at both the species and strain levels. All the primers tested, except the M13 core sequence, generated unique patterns with most strains. The results indicate the usefulness of PCR analysis with primer M13 for identification of the *H. anomala* species. The screening of CBS (Utrecht) collection strains of *H. anomala* showed that they are rather difficult objects for genetic hybridization analysis. The strains have low fertility, viz. very poor sporulation, low mating type activities and, as a rule, non-viable ascospores. The majority of the hybrids obtained are polyploid, probably tetraploids, as judged by the segregation of control auxotrophic markers. Nevertheless, some monosporic cultures of the strains studied, including the biocontrol yeast J121, formed diploid hybrids with regular meiotic segregation of control auxotrophic markers. As a rule, *H. anomala* isolates are homothallic, showing a delayed self-diploidization. Rare stable heterothallic strains of *H. anomala* also occur.

9. Naumov G.I., Naumova E.S., Korshunova I.V., Jakobsen M. 2001. Genetic identification of cultured *Saccharomyces* yeasts from Africa and Asia. (in preparation).

Using genetic hybridization analysis, we reidentified 16 *Saccharomyces* sensu stricto strains isolated from indigenous foods in Africa and Asia as *S. cerevisiae*. The molecular karyotypes of the strains were also determined. The fertile genetic lines created are of interest for evolutionary genetics and breeding programs.

IV. Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Canada S7N 5A8. Communicated by W. M. (Mike) Ingledew <ingledew@sask.usask.ca>.

The following papers have been published since our last report.

1. W. M. Ingledew. Alcohol production by *Saccharomyces cerevisiae*: a yeast primer. Chapter 5. The Alcohol Textbook (3rd edition). 1999. Edited by: K.A. Jacques, T.P. Lyons, and D.R. Kelsall. Nottingham University Press.

2. N. V. Narendranath, K.C. Thomas and W.M. Ingledew. 2000. Urea hydrogen peroxide reduces the numbers of lactobacilli, nourishes yeast, and leaves no residue in the ethanol fermentation. Appl. Environ. Microbiol. **66**:4187-4192.

Urea hydrogen peroxide (UHP) at a concentration of 30 - 32 mmoles/L reduced the numbers of five lactobacilli (*L. plantarum, L. paracasei, Lactobacillus* #3, *L. rhamnosus* and *L. fermentum*) from ~10^7 CFU/ml to ~10^2 CFU/ml in a 2 h preincubation at 30° C of normal gravity wheat mash of ~21 g/100 ml dissolved solids containing normal levels of suspended grain particles. Fermentation completed 36 h after inoculation of *Saccharomyces cerevisiae* in the presence of UHP even when wheat mash was deliberately contaminated (infected) with *L. paracasei* at ~10^7 CFU/ml. There were no significant differences in the maximum ethanol produced between treatments where urea hydrogen peroxide was used to kill the bacteria and controls (where no bacteria were added). However, the presence of *L. paracasei* at ~10^7 CFU/ml without added agent resulted in a 5.84% reduction in the maximum ethanol produced as compared to the control. The bactericidal activity of UHP is greatly affected by the presence of particulate matter. In fact, only 2 mmoles/L urea hydrogen peroxide were required for disinfection when mashes had little or no particulate matter present. No significant differences were observed in the decomposition of hydrogen peroxide in normal gravity wheat mash at 30° C whether the bactericidal agent was added as H_2O_2 or as urea hydrogen peroxide. NADH peroxidase activity (involved in degrading H_2O_2) increased significantly ($P = 0.05$) in the presence of 0.75 mM hydrogen peroxide (sub-lethal level) in all the five strains of lactobacilli tested, but did not persist in cells regrown in the absence of H_2O_2. H_2O_2-resistant mutants were not expected or found when lethal levels of H_2O_2 or UHP were used. Contaminating lactobacilli can be effectively managed by UHP - a compound which when used near 30 mmoles/L happens to provide near optimum levels of assimilable nitrogen and oxygen that aid in vigorous fermentation performance by yeast.

3. N. V. Narendranath, K.C. Thomas and W.M. Ingledew. 2001. Effects of acetic acid and lactic acid on the growth of *Saccharomyces cerevisiae* in a minimal medium. J. Indust. Microbiol. Biotechnol. **26**:171-177.

Specific growth rates (μ) of two strains of *Saccharomyces cerevisiae* decreased exponentially ($R^2 > 0.9$) as the concentrations of acetic acid or lactic acid were increased in minimal media at 30° C. Moreover, the length of the lag phase of each growth curve (h) increased exponentially as increasing concentrations of acetic or lactic acid were added to the media. The minimum inhibitory concentration (MIC) of acetic acid for yeast growth was 0.6 % w/v (100 mM) and that of lactic acid was 2.5 % w/v (278 mM) for both strains of yeast studied. However, acetic acid at concentrations as low as 0.05 - 0.1 % w/v and lactic acid at concentrations of 0.2 - 0.8 % w/v begin to stress the yeasts as seen by reduced growth rates, and decreased rates of glucose consumption and ethanol production as acetic or lactic acid in the media were raised. In the presence of increasing acetic acid, all the glucose in the medium was eventually consumed even though the rates of consumption differed. However, this was not observed in the presence of increasing lactic acid where the glucose consumption was extremely protracted even at a concentration of 0.6 % w/v (66 mM). Response surface central composite design was used to evaluate the interaction between acetic and lactic acids on the specific growth rate of both yeast strains at 30° C. The data was analysed using the general linear models (GLM) procedure. From the analysis, the interaction between acetic acid and lactic acid was statistically significant ($P \leq 0.001$), i.e. the inhibitory effect of the two acids when present together in a medium is highly synergistic.

4. K.C. Thomas, S. H. Hynes and W.M. Ingledew. 2001. Effect of lactobacilli on yeast growth, viability, and batch and semi-continuous alcoholic fermentation of corn mash. J. Appl. Microbiol. **90**:819-828.

Aim: The aim of this study was to evaluate interactions between *Saccharomyces cerevisiae* and selected strains of lactobacilli regarding cell viabilities, and production of organic acids and ethanol during fermentation. Methods and Results: Corn mashes were inoculated with yeasts and selected strains of lactobacilli, and fermented in batch or semi-continuous (cascade) mode. Ethanolic fermentation rates and viabilities of yeast were not affected by lactobacilli unless the mash was pre-cultured with lactobacilli. Then, yeast growth was inhibited and the production of ethanol was reduced by as much as 22%. Conclusion: Yeasts inhibited the multiplication of lactobacilli and this resulted in reduced production of acetic and lactic acids. The self-regulating nature of the cascade system allowed the yeast to recover, even when the lactobacilli had a head start, and reduced the size of the population of the contaminating Lactobacillus to a level which had an insignificant effect on fermentation rate or ethanol yield. Significance and Impact of the Study: Contamination during fermentation is normally taken care of by the large yeast inoculum, although yeast growth and fermentation rates could be adversely affected by the presence of high numbers of lactobacilli in incoming mash or in transfer lines.

V. Institute of Microbiology, Academy of Sciences of Czech Republic, Vídenská 1083, CZ 142 20 Praha 4, Czech Republic. Communicated by J. Vorísek <vorisek@biomed.cas.cz>.

Recent publication.

1. J. Vorísek. 2000. Functional morphology of the secretory pathway organelles in yeast. Microscopy Res. Technique **51**:530-546.

The glycoprotein secretory pathway of yeast serves mainly for cell surface growth and cell division. It involves a centrifugal transport of transit macromolecules among organelles, whose membranes contain resident proteins needed for driving the transport. These resident membrane proteins return by retrograde vesicular transport. Apart from this, the pathway involves endocytosis. The model yeast *Saccharomyces cerevisiae* and vertebrate cells were found to contain very similar gene products regulating the molecular mechanism of glycoprotein transport, and the cellular mechanism of their secretion pathways was therefore also presumed to be identical. Biochemists have postulated that, in *S. cerevisiae*, the translocation of peptides through the endoplasmic reticulum membranes into the lumen of ER cisternae and the core glycosylation is followed by a vector-mediated transport into the functional cascade of the Golgi system cisternae and between them. This is the site of maturation and sorting of glycoproteins, before the ultimate transport by other vectors involving either secretion from the cells (exocytosis across the plasmalemma into the cell wall) or transport into the lysosome-like vacuole via a prevacuolar compartment, which serves at the same time as a primary endosome. The established cellular model of secretion deals with budding yeast; interphase yeast cells, in which the secretion is limited and which predominate in exponential cultures, have not been taken into consideration. The quality of organelle imaging in *S. cerevisiae* ultra-thin sections depends on the fixation technique used and on specimen contrasting by metals. The results achieved by combinations of different techniques differ mostly in the imaging of bilayers of membrane interfaces and the transparence of the matrix phase. Fixation procedures are decisive for the results of topochemical localisations of cellular antigenic components or enzyme activities, which form the basis of the following survey of functional morphology of organelles involved in the yeast secretory pathway. The existing results of these studies do not confirm all aspects of the vertebrate model of the Golgi apparatus proposed by molecular geneticists to hold for *S. cerevisiae*, and alternative models of the cellular mechanism of secretion in this yeast are therefore also discussed.

VI. Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502-8585, Japan. Communicated by M. Miyata <miyata@gifu-pu.ac.jp>.

Recent publication.

1. M. Miyata, H. Miyata, B.F. Johnson. 2000. Sibling differences in cell death of the fission yeast, *Schizosaccharomyces pombe*, exposed to stress conditions. Antonie van Leeuwenhoek, **78**:203-207.

Selective death of fission yeast cells of five strains was observed: one sibling, of a V-pair formed at fission, died while the other survived after being transferred to extreme or sub-lethal conditions (from pH 4.5 to pH 7.0, from 32°C to 41°C, or to medium containing acridine orange, 200µg/ml). Death occurred preferentially to the sib with more fission scars or to that sib with new end derived from its mother cell which had actively grown in length. Thus, the differences between V-pair siblings in stress response related to morphology derived from fission scars and extension growth. From these observations, we rationalize the meaning of aging and rejuvenation in a yeast population with relation to scars and growth in the fission yeast *Schizosaccharomyces pombe.*

VII. School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England. Communicated by J.A. Barnett <J.Barnett@uea.ac.uk>.

Current publications.

1. J.A. Barnett and F.W. Lichtenthaler. 2001. A history of research on yeasts 3: Emil Fischer, Eduard Buchner and their contemporaries, 1880-1900. Yeast **18**:363-388.

2. In preparation: A history of research on yeasts 4: cytology.

VIII. Laboratorium voor Microbiologie, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands. Communicated by W.J. Middelhoven <Wout.Middelhoven@algemeen.micr.wau.nl>

The following papers appeared recently.

1. W.J. Middelhoven, E. Guého and G.S. de Hoog. 2000. Phylogenetic position and physiology of *Cerinosterus cyanescens*. Antonie van Leeuwenhoek **77**:313-320.

Cerinosterus cyanescens is a pathogenic yeastlike fungus that is isolated from the clinical environment but rarely from natural habitats such as plants. Partial 25S rRNA sequencing of *C. cyanescens* showed it to be a close relative of *Microstroma juglandis*, a member of the basidiomycetous order *Microstromatales*. It is unrelated to the generic type species, *C. luteoalba*, which is a member of the order *Dacrymycetales*. The clinical occurrence of *C. cyanescens* is possibly explained by its thermotolerance and lipolytic activity. The species' nutritional profile is established. Growth on n-hexadecane is rapid. It grows well on typical plant constituents such as gallic, tannic, vanillic and p-coumaric acids, but not on 3-hydroxybenzoic acid, phenol of hydroquinone. The failure to assimilate D-galactose, L-sorbose and ethylamine, the presence of urease and sensitivity to cycloheximide are diagnostic for the species.

2. W.J. Middelhoven. Biochemical abilities of yeasts growing on plants in an arid climate. Abstracts 7th International Symposium on the Microbiology of Aerial Plant Surfaces (Phyllosphere 2000), University of California, Berkeley, August 2000.

This abstract is a brief summary of a paper that appeared in Antonie van Leeuwenhoek 72(1997)81-89.

3. W.J. Middelhoven and C.P. Kurtzman. Correlation between phylogeny and physiology in yeasts. Abstract Tenth International Symposium on Yeasts, Arnhem, The Netherlands, August-September 2000.

This abstract deals with physiological relationships of phylogenetically related ascomycetous yeast species. From literature data it appears that the great majority of methanol-assimilating yeast species are found in one clade. Crabtree-positive, fermentative yeast species are distributed over the *Saccharomyces* clade, the *Dekkera* clade and the

Schizosaccharomyces clade, as well as some phylogenetically unrelated species. A clade consisting of species of the genera *Stephanoascus, Arxula* and *Blastobotrys* is notable for assimilation of n-hexadecane, putrescine, adenine, uric acid, glycine, isobutanol, leucine and isoleucine as sole sources of carbon and energy. The ability to assimilate at least 7 of these 8 compounds is characteristic of this clade, of *Candida blankii* and of two Euascomycetes, viz. *Emericella nidulans* and *Endomyces scopularum*.

4. W.J. Middelhoven. 2000. Identification of yeasts present in sour fermented foods and fodders. In: J.F.T. Spencer and A.L. Ragout de Spencer (Eds.) Food Microbiology Protocols, Methods in Biotechnology, Vol. 14, Humana Press Inc., Totowa, New Jersey, U.S.A., 2000, pp. 209-224.

An identification key to 50 yeast species known to be present in foods and fodders that underwent a lactic acid fermentaion is provided. It is based on easy and rapid methods including light microscopy, the API ID32C identification test system, assimilation of nitrate and ethylamine as nitrogen sources, fermentative ability, vitamin requirement and maximum growth temperature.

5. M.H.M. Eppink, E. Cammaart, D. van Wassenaar, W.J. Middelhoven and W.J.H. van Berkel. 2000. Purification and properties of hydroquinone hydroxylase, a FAD-dependent monooxygenase involved in the catabolism of 4-hydroxybenzoate in *Candida parapsilosis* CBS 604. European J. Biochem. **267**:6832-6840.

Candida parapsilosis catabolizes 4-hydroxybenzoate through the initial formation of hydroquinone (1,4-dihydroxybenzene). High levels of hydroquinone hydroxylase are induced when the yeast is grown on either 4-hydroxybenzoate, 2,4-dihydroxybenzoate, 1,3-dihydroxybenzoate or hydroquinone as the sole carbon source. The monooxygenase constitutes up to 5% of the total amount of protein and was purified to apparent homogeneity in three chromatographic steps. It is a homodimer of about 150 kDa with each subunit containing a tightly noncovalently bound FAD. The enzyme catalyzes the ortho-hydroxylation of a wide range of monocyclic phenols with the stoechiometric consumption of NADPH and oxygen. The reaction product of hydroquinone is 1,2,4-trihydroxybenzene that subsequently is subject to ring opening by a dioxygenase. N-terminal peptide sequence analysis revealed structural relationship to the phenol hydroxylase of *Trichosporon cutaneum*.

6. W.J. Middelhoven. 2001. Identification of pathogenic *Trichosporon* species. Abstract Spring Meeting of the Dutch Society for Medical Microbiology, Arnhem, The Netherlands, Nederl. Tijdschr. Med. Microbiol. Supplement to Vol. 9, March 2001.

An identification key to all species of the genus *Trichosporon* able to grow at 37°C is provided. These species include pathogens and species suspected to be pathogen. The characteristics used in the key include assimilation of uric acid, of ethylamine, of tyramine, of L-phenylalanine and of L-4-hydroxyproline as sole sources of carbon and nitrogen, of rhamnose, melezitose, quinate pH 5.5, 2,3-dihydroxybenzoate, orcinol and 4-ethylphenol as sole carbon sources, and the maximum growth temperature.

7. W.J. Middelhoven, G. Scorzetti and J.W. Fell. 2001. *Trichosporon porosum* comb. nov., am anamorphoc basidiomycetous yeast inhabiting soil, related to the *loubieri/laibachii* group of species that assimilate hemicelluloses and phenolic compounds. FEMS Yeast Research **1**:1-8. (Available at: www.fems-microbiology.org).

Several isolates representing the genus *Trichosporon* were collected from soil in The Netherlands. Based on classical growth tests these strains were identical. Polygalacturonate, xylan and several phenolic compounds were also assimilated, characteristics with potential ecological importance in soil habitats. Molecular analysis of the D1/D2 region of the 26S rDNA confirmed identity with the type strain (CBS 2040) of *Apiotrichum porosum* Stautz (1931) that, however, showed a different morphology. The new combination *Trichosporon porosum* is presented. Strains of *T. porosum* showing a morphology more characteristic of the genus are CBS 8396, 8397 and 8522. Based on molecular sequence analysis, *T. porosum* may be related to *T. sporotrichoides*, within a weakly related clade that includes species such as *T. laibachii* and *T. loubieri.* Characters distinguishing the nine species of the *laibachii/loubieri* group of species were listed. These include traditionally used tests as well as assimilation patterns of some aliphatic and phenolic compounds. Based on these tests, *T. laibachii* and *T. multisporum* could be separated. By other authors these are considered to be conspecific.

XIX. Department of Genetics, University of Debrecen, POBox 56, H-4010 Debrecen, Hungary. Communicated by M. Sipiczki.

Recent publications.

Full papers.

1. Sipiczki, M., Yamaguchi, M., Grallert, A., Takeo, K., Zilahi, E., Bozsik, A., Miklos, I. 2000. The role of cell shape in the determination of division plane in *Schizosaccharomyces pombe*: random orientation of septa in spherical cells. J. Bacteriol. **182**:1693-1701, 2000.

2. Zilahi, E., Miklos, I., Sipiczki, M. 2000. The *Schizosaccharomyces pombe sep15*$^{+}$ gene encodes a protein homologous to the Med8 subunit of the *Saccharomyces cerevisiae* transcriptional mediator complex. Curr. Genet. **38**:227-232.

3. Zilahi, E., Salimova, E., Simanis, V., Sipiczki, M 2001. The *S. pombe sep1* gene encodes a nuclear protein that influences the expression of the *cdc15* gene. FEBS Letters **481**:105-108.

4. Sipiczki, M., Bozsik, A. 2000. The use of morphomutants to investigate septum formation and cell separation in *Schizosaccharomyces pombe*. Arch. Microbiol. **174**:386-392.

5. Molnar, M., Parisi, S., Kakihara, Y., Nojima, H., Yamamoto, A., Hiraoka, Y., Bozsik, A., Sipiczki, M., Kohli, J. 2001. Characterization of *REC7*, an early meiotic recombination gene in *Schizosaccharomyces pombe*. Genetics **157**:519-532.

6. Sipiczki, M., Romano, P., Lipani, G., Miklos, I., Antunovocs, Z. 2001. Analysis of yeasts derived from natural fermentation in a Tokaj winery. Antonie van Leeuwenhoek **79**:97-105.

7. Sipiczki, M., Grallert, A., Zilahi, E., Miklos, I., Szilagyi, Z. 2001. Multifunctional cytokinesis genes in *Schizosaccharomyces pombe*. Acta Biol. Hung. **52**:133-141.

Review.

8. Sipiczki, M. 2000. Where does fission yeast sit on the tree of life? Genome Biology **1**:1011.1-1011.4.

Chapter in book.

9. Sipiczki, M., Miklos, I., Leveleki, L., Antunovics, Z. 2001. Genetic and chromosomal stability of wine yeasts. In "Food Microbiology Protocols (eds. J.F.T. Spences and A.L. Ragout de Spencer)" Humana Press, Totowa, New Jersey, USA, pp. 273-281.

Abstracts.

10. Bozsik. A., Yamaguchi, M., Takeo, K., Sipiczki, M. 2000. Dimorphism and cell polarity transitions in *Schizosaccharomyces japonicus*: a novel experimental model. 2nd Asia-Pacific Mycological Conference on Biodiversity and Biotechnology. Hongkong, Abstracts p. 78.

11. Sipiczki, M., Antunovics, Z. 2000. Tests for genetic stability in wine yeasts. 2nd Asia-Pacific Mycological Conference on Biodiversity and Biotechnology. Hongkong, Abstracts p. 108.

12. Sipiczki, M., Romano, P., Antunovics, Z. 2000. Characterization of three yeast strains isolated from Tokaj wines. XXVIII Annual Conference on Yeasts. Czechoslovak Society for Microbiology. Smolenice. Programme and Abstracts p. 70.

13. Zilahi, E., Sipiczki, M. 2001. The *Schizosaccharomyces pombe* gene *sep15*$^{+}$ encodes a protein which shows significant homology to Med8, a component of the *Saccharomyces cerevisiae* mediator complex. XXVIII Annual Conference on Yeasts. Czechoslovak Society for Microbiology. Smolenice. Programme and Abstracts p. 71.

X. Alkomohr Biotech Ltd., Viikinkaari 9, FIN-00710 Helsinki, Finland. Communicated by M. Korhola <matti.korhola@yeast.pp.fi>.

We have finally completed our `SixPack´ work on the functional analysis of unknown yeast genes under the EUROFAN project.

1. Aittamaa, M., Turakainen, H. and Korhola, M. 2001. Functional analysis of six ORFs from *Saccharomyces cerevisiae* chromosome IV: two-spored asci produced by disruptant of YDR027c and strain dependent DNA heterogeneity around YDR036c. Yeast (in press).

Six *S. cerevisiae* FY1679 deletion mutants were made by replacing six open reading frames (ORFs) of the chromosome IV right arm with *kanMX4* selection marker. No essential genes were found. The basic phenotypic analysis showed that the haploid and homozygous deletants for the ORF YDR027c (*LUV1, VSP54* or *RKI1*) grew slowly. The diploid homozygous deletants had a low frequency of sporulation. They produced asci with no more than one or two haploid spores. The majority of these spores formed was not viable. The deletion of the other ORFs, YDR022c (*CIS1*), YDR030c (*RAD28*), YDR032c (*PST2*), YDR033w (*MRH1*) and YDR036c, did not change the phenotypes tested in strains FY1679 or CEN.PK2. This work showed some differences in the DNA between FY1679 and CEN.PK2: the upstream regions of YDR036c in these two strains are too different to hybridise properly preventing YDR036c deletion by recombination in the CEN.PK2 background and in addition, there are different sets of transposable elements on the other side of the ORF.

XI. Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany. Communicated by G. Kunze <kunzeg@ipk-gatersleben.de>.

Recent publications.

1. K. Tag, K. Riedel & G. Kunze. 2000. Yeast based sensors for environmental control. Recent Res. Bioenergetics (in press).

Microbial sensors are suitable for the monitoring of complex environmental parameters such as BOD, pollutants and heavy metals, which is difficult or impossible to achieve with enzyme sensors. One prerequisite for these measurements is microorganisms with a wide substrate spectrum. In order to find the most suitable organism, many microorganisms were physiological characterised by the sensor technique. By this procedure the non-conventional yeast *Arxula adeninivorans* has been identified as a top favourite for the BOD-sensor. The novel *Arxula*-sensor based on budding cells or mycelia of this dimorphic yeast is one of the most suitable sensors for rapid measurement of biodegradable substances. With their application the environmental monitoring with microbial sensors could be improved. Very short response times, a serial coefficient of ±5%, high measuring ranges and operational stability enables *Arxula* budding cell- and mycelia-sensors to monitor sea and other salty waters. The possibility to detect only a specific substance by microbial sensors is given by a sensor which measured Cu^{2+} by an amperometric method. This sensor is suitable to measure this heavy metal ion concentrations in wastewater contaminated with high Cu^{2+} concentrations such as wastewaters from the galvanic industry and computer hardware factories. So an apparatus with various microbial sensors can be used to measure sensorBOD and Cu^{2+}.

XII. Center for Process Biotechnology, BioCentrum - DTU, Building 223, The Technical University of Denmark, DK-2800 Lyngby, Denmark. Communicated by L.Olsson <LO@ibt.dtu.dk>.

The research activities on yeast at the Center for Process Biotechnogy combines physiological studies with advanced analytical techniques and mathematical modelling with the objective of increasing our understanding of yeast. The following topics are studied: *Fermentation of complex substrates* (metabolic engineering of the galactose and the xylose metabolism, mixed sugar utilisation, glucose repression and fermentation inhibitors). *Yeast physiology* (pyruvate metabolism in *Saccharomyces kluyveri,* modelling of the pyruvate node, transcriptome analysis of *Saccharomyces cerevisiae,* redox metabolism). *Metabolic network analysis* (futile cycles, functional genomics). *Analytical biotechnology* (measurement of intracellular metabolites, multiwave-length flourescence, CE and combination sensors).Recent publications.

1. A. K. Gombert; M. M. dos Santos; B. Christensen; J. Nielsen. 2001. Network identification and flux quantification in the central metabolism of *Saccharomyces cerevisiae* at different conditions of glucose repression. J. Bacteriol. **183**:1441-1451.

2. A. K. Gombert; J. Nielsen. 2000. Mathematical modelling of metabolism. Curr. Opinion Biotechnol. **11**:180-186.

3. K. Møller, L. Olsson, and J. Piškur. 2001. Ability for anaerobic growth is not sufficient for development of the petite phenotype in *Saccharomyces* yeasts. J. Bacteriol. **183:**2485-9.

Saccharomyces cerevisiae is a petite-phenotype-positive ("petite-positive") yeast, which can successfully grow in the absence of oxygen. On the other hand, *Kluyveromyces lactis* as well as many other yeasts are petite negative and cannot grow anaerobically. In this paper, we show that *Saccharomyces kluyveri* can grow under anaerobic conditions, but while it can generate respiration-deficient mutants, it cannot generate true petite mutants. From a phylogenetic point of view, *S. kluyveri* is apparently more closely related to *S. cerevisiae* than to *K. lactis*. These observations suggest that the progenitor of the modern *Saccharomyces* and *Kluyveromyces* yeasts, as well as other related genera, was a petite-negative and aerobic yeast. Upon separation of the *K. lactis* and *S. kluyveri-S. cerevisiae* lineages, the latter developed the ability to grow anaerobically. However, while the S. kluyveri lineage has remained petite negative, the lineage leading to the modern *Saccharomyces* sensu stricto and sensu lato yeasts has developed the petite-positive characteristic.

4. K. Møller, L. D. Tidemand, J. R. Winther, L. Olsson, J. Piškur, and J. Nielsen. 2001. Production of a heterologous proteinase A by *Saccharomyces klyveri.* Appl. Microbiol. Biotecnol. In press.

5. J. Nielsen. 2001. Metabolic engineering. Appl. Microbiol. Biotechnol. **55:**263-283.

6. T. L. Nissen; M. Anderlund; J. Nielsen; J. Villadsen; M. C. Kielland-Brandt. 2001. Expression of a cytoplasmic transhydrogenase in *Saccharomyces cerevisiae* results in formation of 2-oxoglutarate due to depletion of the NADPH pool. Yeast **18**:19-32.

7. S. Ostergaard; L. Olsson; M. Johnston; J. Nielsen. 2000. Increasing galactose consumption by *Saccharomyces cerevisiae* through metabolic engineering of the *GAL* gene regulatory network. Nature Biotechnol. **18**:1283-1286.

8. S. Ostergaard; L. Olsson; J. Nielsen. 2001. *In vivo* dynamics of galactose metabolism in *Saccharomyces cerevisiae*: Metabolic fluxes and metabolite levels. Biotechnol. Bioeng. In press.

9. S. Ostergaard; K. O. Walløe; C. S. G. Gomes; L. Olsson; J. Nielsen. 2001. The impact of *GAL6*, *GAL80*, and *MIG1* on glucose control of the *GAL* system in *Saccharomyces cerevisiae*. FEMS Yeast Research. In press.

10. Zaldivar, J., Nielsen, J. and L. Olsson. 2001. Metabolic engineering for fuel ethanol production. Appl. Microbiol. and Biotechnol. In press.

XIII. Institute of Microbiology & Biotechnology, University of Latvia, Kronvald blvd., 4, Riga LV-1586, Latvia. Communicated by O. Muter <muter@latnet.lv>.

The following is a summary of our research on the sorption activity of intact and dehydrated *C .utilis* cells and their physiological response to combined stress, with relevant publications.

The application of fungi and yeasts in heavy metal recovery from aqueous solutions is under intensive investigation during last years. Biomass dehydration is considered as a promising way to increase its sorption activity, besides it can improve the storage and transportation of biosorbent. Our previous results showed that dehydrated *C.utilis* has higher Cr(VI) sorption activity comparing with intact one, therefore yeast *C.utilis* was chosen as a model organism for our experiments on the mechanisms of Cr(VI) sorption by intact and dehydrated yeast biomass [1,2].

Yeast physiological state is one of the most important factors which influence metal sorption activity. Since cell wall structure and the metabolism in the cell depend on substrate composition, growth in different media should influence the capacity and selectivity of metal uptake by creating other binding sites or diverse enzymatic system within the cell. The Cr(VI) sorption by intact and dehydrated *C.utilis*, pre-grown in the different medium to the different phase of growth, was observed [3]. A decrease of sorption activity in a range [molasses > ethanol > glucose] was revealed as for intact as for dehydrated cells, as well as for exponentially and stationary grown culture. An exception was the *C.utilis* cells, grown in the medium with glucose to the exponential phase of growth, which demonstrated considerably higher sorption activity in the intact state compared with *C.utilis* culture in another physiological states. Addition of cysteine to the growth medium with glucose resulted in enhanced Cr(VI) sorption by intact and dehydrated *C.utilis* cells. A possible correlation of the final medium pH and Cr(VI) sorption activity was revealed, i.e. for the cells grown with glucose to the exponential phase (pH 2.9) and for the cells grown with glucose and cysteine to the stationary phase (pH 2.7). The final

medium pH in another samples, demonstrated the lower Cr(VI) sorption activity, was in the range 4.2-4.6. Comparative analysis of the main cell structural components, using the infrared spectroscopy, has revealed a tendency of lipid component to decrease in the cells with enhanced Cr(VI) sorption capacity in the intact state, e.g. *C.utilis* exponentially grown with glucose. Comparison of nine yeast strains belonging to the different species and genera showed, that yeast dehydration not always lead to the activation of their Cr(VI) sorption activity [3].

Since real wastewater contains all kinds of components of pollution, multicomponent systems need further detailed study. The interactive effects of a mixture on the cell are extremely complex and depend on the number of metals competing for binding sites, combination and concentration of metals, order of metal addition, residence time and test criterion. In our experiments the Cr(VI) sorption by intact and dehydrated *Candida utilis* was also studied in the presence of other metals, in particular, Cu(II), Zn(II), Cd(II), Pb(II). The anion $[Cr_2O_7]^{2-}$ and cation Me^{2+} sorption kinetics was investigated both in single- and dual-metal situation [4].

Heavy metals can significantly change a physiological state of biomass during sorption process. In the experiments with dehydrated yeasts incubated with metals, the cells bear at least stress factors, i.e. dehydration, fast rehydration and a presence of heavy metal in rehydration solution. In summary, the toxicity range for intact *C.utilis* under studied conditions was as follows: Cu > Cr > Pb > Cd = Zn (initial metal conc. 500 and 1000 mg Me l^{-1}; period of incubation 24 h). Discussing the results which were obtained in the experiments with dehydrated cells, it is necessary to note, that only of 50 % cells in the tested population remain viable after dehydration and rehydration processes. It was shown that a resistance of dehydrated-rehydrated cells to Pb, Zn, and Cd was decreased, whereas a resistance to Cr was noticeably increased compared to the intact cells. The mechanisms of an enhanced Cr resistance of dehydrated yeast cells are still unknown.

An effect of chromium(VI) ions on the growth and bioaccumulation properties of growing cells *Candida utilis* was studied. Molasses media for yeast growth containing 20 g glucose l^{-1} and 50-500 mg Cr(VI) l^{-1} were used in batch cultivation. Addition of 100 mg Cr(VI) l^{-1} resulted in a 3-fold decrease in the cell concentration, as compared to the culture grown without metal. Cr(VI) inhibited culture growth in a concentration-dependent manner, this dependence having not been found linear. Glucose consumption by growing cell was found to depend on the initial Cr(VI) concentration in the medium and correlated with the growth activity. No inhibitory effect of high Cr(VI) concentrations on the activity of some exo-enzymes of *C.utilis* cells was observed. During *C.utilis* cultivation Cr(VI) was found to be partially reduced to an ultimate Cr(III) in all tested ranges of the Cr(VI) concentrations. The specific chromium uptake by cells was detected in biomass which grew in the presence of 100-300 mg Cr(VI) l^{-1} in a period of 48 h-96 h. The highest values were achieved after 96 h growth in the presence of 200 and 300 mg Cr(VI) l^{-1} and were 7.3 and 7.2 mg Cr g dw^{-1}, respectively. Electron microscopic observations showed morphological changes in yeast cells to be more pronounced upon culture growth with 100-300 mg Cr(VI) l^{-1} than in the cells subjected to higher metal concentrations. The conclusion has been made that the mechanism of the Cr(VI) toxic effect on the growing yeast cells can vary in dependence of the metal concentration [5].

Besides, a toxic effect of Zn, Cd, Cu, Pb was studied for the growing *Candida utilis*, using an inhibition of growth and metal uptake by cells as test criteria. Thus, the sequence in decreasing order of metal toxicity for the growing cells in the molasses medium was as following: Cd > Cr > Zn ≥ Cu ≥ Pb. The metal uptake by cells after 48 h cultivation can be presented in the range: Pb > Cu > Zn > Cr > Cd. The specific metal uptake, determined as the amount of metal per unit of dry weight of cells was as follows: Pb > Cd > Cr > Cu > Zn. Cell concentration and metal concentration were determined after 48 h cultivation in molasses medium with initial metal concentration 200 mg Me l^{-1}. In our experiments the presence of Cu and Zn in the concentration 200 mg Me l^{-1} did not reveal any considerable inhibitory effect to the growth of *C.utilis* culture. Nevertheless, morphological changes of cells (cell grouping in chains), cultured in the presence of Cu, can indicate to the significant changes in the cell. The effect of Pb to the growing *C.utilis* cells, in our view, deserves to be studied in further investigations. Among all tested metals the lead only was accumulated by growing cells in great quantities (63 %) without any detectable changes in the growth activity.

A conclusion could be made, that a search of appropriate growth conditions and specific cell treatment can noticeably optimise the heavy metal sorption by yeast biomass. Further investigations, in our view, have to be focused on the economicaly feasible technological solutions, which will take into account the results presented above.

Resting and growing cells demonstrated completely different mechanisms of resistance to acute and chronic metal stress, correspondingly. Obviously, a comparative study of the cell response to the metal stress on the physiological level could i) provide information on a quantitative toxicity evaluation of the metals; ii) reveal some metabolic and (ultra)structural changes, being a sequence of the main self-protective mechanisms of eucaryotic cell.

1. Rapoport A., Muter O. 1995. Biosorption of hexavalent chromium by yeasts. Process Biochemistry **30**(2):145-149.

2. Muter O., Ventina, Patmalnieks A., Millers D., Grigorjeva L., Rapoport A. 2000. Sorption activity of yeasts and their physiological response to combined stress. In: The rising power of yeasts in science and industry. 10th Int.Symp. on Yeasts, ISY 2000, Symposium Book, p.372.

3. Muter O., Grube M., Rapoport A. 2000. Effect of growth conditions to the Cr(VI) sorption by intact and dehydrated *Candida utilis*. In: Future trends in the food and nutrition development. Int.Conf. 2001, Jelgava, Latvia, 177-186.

4. Muter O., Lubinya I., Millers D., Grigorjeva L., Ventinya E., Rapoport A. Cr(VI) sorption by intact and dehydrated *Candida utilis* in the presence of other metals. Submitted.

5. O.Muter, A.Patmalnieks, A.Rapoport. 2001. Interrelations on the yeast *Candida utilis* and Cr(VI): metal reduction and its distribution in the cell and medium. Process Biochemistry **36**(10):963-970.

XIV. CREM – Centro de Recursos Microbiológicos, Secção Autónoma de Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825-114 Caparica, Portugal. Communicated by J. P. Sampaio <jss@mail.fct.unl.pt>.

The following papers, whose abstracts were given in the last issue, have now been published.

1. Sampaio, J.P., Gadanho, M. and Bauer, R. 2001. Taxonomic studies on the genus *Cystofilobasidium*: description of *Cystofilobasidium ferigula* sp. nov. and clarification of the status of *Cystofilobasidium lari-marini.* Int. J. Syst. Evol. Microbiol. **51**:221-229.

2. Sampaio, J.P., Gadanho, M., Santos, S., Filomena, L. D., Pais, C., Fonseca, Á. and Fell, J.W. 2001. Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *R. kratochvilovae* and related anamorphic species. Int. J. Syst. Evol. Microbiol. **51**:687-697.

The following paper was recently published.

3. Gadanho, M., Sampaio, J.P. and Spencer-Martins, I. 2001. Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *R. azoricum* sp. nov. Can. J. Microbiol. **47**: 213-221.

This report presents the description of a new heterothallic *Rhodosporidium* species, *R. azoricum* sp. nov. The new species is based on two strains previously identified as *Rhodotorula glutinis*, which were isolated from soil in São Miguel island, Azores, Portugal. Evidence that the two strains were conspecific and distinct from *Rh. glutinis* was obtained in DNA fingerprinting experiments using the microsatellite-primed PCR approach (MSP-PCR) and the primers M13 and $(GTG)_5$. In order to determine the phylogenetic position of the new species, the nucleotide sequence of the D1/D2 region of the 26S rDNA was analysed and *R. azoricum* was found to belong to a cluster including also *R. fluviale, R. lusitaniae, Sporidiobolus microsporus* and *S. ruineniae.* The life cycle of *R. azoricum* was investigated and comparisons integrating physiological, morphological and molecular data were made with related species.

The following paper was accepted for publication.

4. Kirschner, R., Sampaio, J.P., Gadanho, M., Weiss, M. and Oberwinkler, F. *Cuniculitrema polymorpha* (Tremellales, gen. and sp. nov.), a heterobasidiomycete vectored by bark beetles which is the teleomorph of *Sterigmatosporidium polymorphum*. Antonie van Leeuwenhoek.

In a study of the mycobiota associated with bark beetles, a dimorphic fungus producing longitudinally septate basidia of the *Tremella*-type and yeast cells budding off from stalks was collected. Detailed morphological, physiological and molecular studies revealed that this fungus represents the teleomorph of *Sterigmatosporidium polymorphum*. Consequently, a new genus, *Cuniculitrema* gen. nov., and a new species, *C. polymorpha* sp. nov., are proposed. Comparative morphological and molecular studies indicated that the new taxon belongs to a group comprising also species of the stalk-forming anamorphic genera *Fellomyces* and *Kockovaella*. The systematic position of *Cuniculitrema*, its intraspecific variability and the relationship with the genus *Tremella* are also addressed.

XVI. Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada N6A 5B7. Communicated by M. A. Lachance <lachance@uwo.ca>.

Request for reprints: In preparation for my work for the next edition of "The Yeasts, a Taxonomic Study," I would be very grateful if colleagues could send me reprints of their work published since 1990 involving the systematics of yeasts in the following genera, or the ecology of yeasts in general.

The taxa of interest are *Candida*, *Clavispora*, *Kodamaea* (*Pichia ohmeri* and relatives), *Kluyveromyces* and related species, *Metschnikowia*, *Sporopachydermia*, *Starmerella* (*Candida bombicola* and relatives), and *Wickerhamiella*.

The following papers, whose abstracts appeared in the last issue, have now appeared in print.

1. Lachance, M.A., J.M. Bowles, S. Kwon, G. Marinoni, W.T. Starmer, and D.H. Janzen. 2001. *Metschnikowia lochheadii* and *Metschnikowia drosophilae*, two new yeast species isolated from insects associated with flowers. Can. J. Microbiol. **47**:103-109.

2. Lachance, M.A., Kaden, J.E., Phaff, H.J., and Starmer, W.T. 2001. Phylogenetic structure of the *Sporopachydermia cereana* species complex. Int. J. Syst. Evol. Microbiol. **51**:237-247.

3. Lachance, M.A., J.M. Bowles, M.M. Chavarria Diaz, and D.H. Janzen. 2001. *Candida cleridarum*, *Candida tilneyi*, and *Candida powellii*, three new yeast species isolated from insects associated with flowers. Int. J. Syst. Evol. Microbiol. **51**:1201-1207.

4. Starmer, W.T., H.J. Phaff, P.F. Ganter, and M.A. Lachance. 2001. *Candida orba* sp. nov., a new cactus-specific yeast species from Queensland, Australia. Int. J. Syst. Evol. Microbiol. **51**:699-705.

5. Lachance, M.A. W.T. Starmer, C.A. Rosa, J.M. Bowles, J.S.F. Barker, and D.H. Janzen. 2001. Biogeography of the yeasts of ephemeral flowers and their insects. FEMS Yeast Research **1**:1-8.

The following papers were accepted recently.

6. Lachance, M.A., J.A. Klemens, J.M. Bowles, and D.H. Janzen. 2001. The yeast community of sap fluxes of Costa Rican *Maclura (Chlorophora) tinctoria* and description of two new yeast species, *Candida galis* and *Candida ortonii*. FEMS Yeast Research **1**: in press.

We report on the yeast community associated with sap fluxes of *Maclura tinctoria*, family Moraceae, in the dry forest of the Area de Conservación Guanacaste, Costa Rica. Eleven samples yielded 7 hitherto undescribed ascomycetous yeasts in the genera *Candida* and *Myxozyma*. We describe the two most abundant as new species. *Candida galis* utilizes very few carbon compounds limited to some alcohols and acids. Analysis of rDNA sequences suggest that it occupies a very basal position among yeasts of ascomycetous affinity. *Candida ortonii* is also restricted in nutritional breath, and growth is generally very slow. It is a sister species to *Candida nemodendra*. The type cultures are: *Candida galis,* strain UWO(PS)00-159.2 = CBS 8842; and *Candida ortonii*, strain UWO(PS)00-159.3 = CBS 8843.

7. Trindade, R.C., J.B. Guerra, M.A. Resende, M.A. Lachance, and C.A. Rosa. 2001. *Candida hagleri*, a new ascomycetous yeast associated with tropical fruits. In press.

Twenty-five strains of the new yeast species *Candida hagleri* have been isolated from frozen pulps of following tropical fruits: acerola (*Malpighia glaba* L.), umbú (*Spondias tuberosa* Avr. Cam.), and mangaba (*Hancornia speciosa* Gom.). *Candida hagleri* was one of the predominant species in the yeast community of these substrates. The new asexual ascomycetous yeast is a sister species of *C. magnoliae* and *C. geochares*, as evidenced by the sequences of the D1/D2 domains of their large subunit ribosomal DNAs. PCR fingerprints using an intron splicing site primer could also separate the three species. The species *C. hagleri* and *C. magnoliae* can be separated on the basis the growth on 50% glucose agar, negative for the first species and positive for the second. The type culture is strain UFMG97- R132 (CBS8824).

VII. Collection de Levures d'Intérêt Biotechnologique (Clib), Laboratoire de Microbiologie et Génétique Moléculaire, INA-PG INRA, BP01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H.-V <clib@grignon.inra.fr>

The following have been recently published.

1. C. Hennequin, A. Thierry, G. F. Richard, G. Lecointre, H. V. Nguyen, C. Gaillardin, B. Dujon. 2000. Microsatellite typing as a new tool for identification of *Saccharomyces cerevisiae* strains. J. Clin. Microbiol. **39**:551-559.

Since *Saccharomyces cerevisiae* appears to be an emerging pathogen, there is a need for a valuable molecular marker able to distinguish among strains. In this work, we investigated the potential value of microsatellite length polymorphism with a panel of 91 isolates, including 41 clinical isolates, 14 laboratory strains, and 28 strains with industrial relevance. Testing seven polymorphic regions (five trinucleotide repeats and two dinucleotide repeats) in a subgroup of 58 unrelated strains identified a total of 69 alleles (6 to 13 per locus) giving 52 different patterns with a discriminatory power of 99.03%. We found a cluster of clinical isolates sharing their genotype with a bakery strain, suggesting a digestive colonization following ingestion of this strain with diet. With the exception of this cluster of isolates and isolates collected from the same patient or from patients treated with *Saccharomyces boulardii*, all clinical isolates gave different and unique patterns. The genotypes are stable, and the method is reproducible. The possibility to make the method portable is of great interest for further studies using this technique. This work shows the possibility to readily identify *S. boulardii* (a strain increasingly isolated from invasive infections) using a unique and specific microsatellite allele.

2. S. Casaregola, H-V Nguyen, G. Lapathitis, A. Kotyk, C. Gaillardin. 2001. Analysis of the constitution of beer yeast genome by PCR, sequencing and subtelomeric sequence hybridization. Int. J. Syst. Evol. Microbiol. (in press).

Lager brewing yeasts, *Saccharomyces pastorianus* (synonym *Saccharomyces carlsbergensis*) are allopolyploid, containing parts of two divergent genomes. *Saccharomyces cerevisiae* contributed to the formation of these hybrids, although the identity of the other species is still unclear. We tested for the presence of alleles specific to *S. cerevisiae* and *S. pastorianus* by PCR/RFLP in brewing yeasts of various origins and in members of the *Saccharomyces sensu stricto* complex. We identified *S. cerevisiae*-type alleles of two genes, *HIS4* and *YCL008c*, in another brewing yeast, *S. pastorianus* CBS1503 (*S. monacensis*), thought to be the source of the other contributor to the lager hybrid. This is consistent with the hybridization of *S. cerevisiae* subtelomeric sequences X and Y' to this strain electrophoretic karyotype. *S. pastorianus* CBS1503 (*S. monacencis*) is therefore probably not an ancestor of *S. pastorianus*, but a related hybrid. *Saccharomyces bayanus*, also thought to be one of the contributor to the lager yeast hybrid, is a heterogeneous taxon containing at least two subgroups, one close to the type strain CBS380^T, the other close to CBS395 (*Saccharomyces uvarum)*. The partial sequences of several genes (*HIS4, MET10, URA3*) were shown to be identical or very similar (over 99%) in *S. pastorianus* CBS1513 (*S. carlsbergensis*), *S. bayanus* CBS380^T and its close derivatives, showing that *S. pastorianus* and *S. bayanus* have a common ancestor. A distinction between two subgroups within *S. bayanus* was made on the basis of sequence analysis: the subgroup represented by *S. bayanus* CBS395 (*S. uvarum*) has 6-8% sequence divergence within the genes *HIS4, MET10* and *MET2* from *S. bayanus* CBS380^T indicating that both *S. bayanus* subgroups diverged recently. The detection of specific alleles by PCR/RFLP and hybridization with *S. cerevisiae* subtelomeric sequences X and Y' to electrophoretic karyotypes of brewing yeasts and related species confirmed our findings and revealed substantial heterogeneity in the genome constitution of Czech brewing yeasts used in production. Sequences are deposited at EMBL under the accession N°: AJ250961, AJ251006, AJ251007, AJ251008, AJ251009, AJ251010, AJ251011, AJ251012, AJ251575, AJ251576.

3. E. Bon, C. Neuvéglise, A. Lépingle, P. Wincker, F. Artiguenave, C. Gaillardin, S. Casaregola. 2000. Genomic exploration of the hemiascomycetous yeasts: *Saccharomyces exiguus.* FEBS Lett. **487**:42-46.

Random sequence tags were obtained from a genomic DNA library of *Saccharomyces exiguus*. The mitochondrial genome appeared to be at least 25.7 kb in size, with a different organization compared to *Saccharomyces cerevisiae*. An unusual putative 953 by long terminal repeated element associated to Ty3 was found. A set of 1451 genes was identified homologous to *S. cerevisiae* open reading frames. Only five gene were identified outside the *S. cerevisiae* taxon, confirming that *S. exiguus* is phylogenetically closely related *to S. cerevisiae*. Unexpectedly, numerous duplicated genes were found whereas they are unique in *S. cerevisiae*. The sequences are deposited at EMBL under the accession numbers: AL407377-AL409955.

4. S. Casaregola, A. Lépingle, E. Bon, C. Neuvéglise, H-V. Nguyen, F. Artiguenave, P. Wincker, C. Gaillardin. 2000. Genomic exploration of the hemiascomycetous yeasts: *Saccharomyces servazzii*. FEBS Lett. **487**:47-51.

The genome of *Saccharomyces servazzii* was analyzed with 2570 random sequence tags totalling 2.3 Mb. BLASTX comparisons revealed a minimum of 1420 putative open reading frames with significant homology to *Saccharomyces cerevisiae* (58% as identity on average), two with *Schizosaccharomyces pombe* and one with a human protein, confirming that *S. servazzii* is closely related to *S. cerevisiae*. About 25% of the *S. servazzii* genes were identified, assuming that the gene complement is identical in both yeasts. *S. servazzii* carries very few transposable elements related to Ty elements in *S. cerevisiae*. Most of the mitochondrial genes were identified in eight contigs altogether spanning 25 kb for a predicted size of 29 kb. A significant match with the *Kluyveromyces lactis* linear DNA plasmid pGKL-1 encoded RF4 killer protein suggests that a related plasmid exists in *S. servazzii*. The sequences have been deposited with EMBL under the accession numbers AL402279AL404848.

5. C. Neuvéglise, E. Bon, A. Lépingle, F. Artiguenave, P. Wincker, C. Gaillardin, S. Casaregola. 2000. Genomic exploration of the hemiascomycetous yeasts: *Saccharomyces kluyveri*. FEBS Lett. **487**:56-60.

The genome of *Saccharomyces kluyveri* was explored through 2528 random sequence tags with an average length of 981 bp. The complete nuclear ribosomal DNA unit was found to be 8656 by in length. Sequences homologous to retroelements of the *gypsy* and *copia* types were identified as well as numerous solo long terminal repeats. We identified at least 1406 genes homologous to *Saccharomyces cerevisiae* open reading frames, with on average 58.1% and 72.4% amino acid identity and similarity, respectively. In addition, by comparison with completely sequenced genomes and the SwissProt database, we found 27 novel *S. kluyveri* genes. Most of these genes belong to pathways or have functions absent from *S. cerevisiae*, such as the catabolic pathway of purines or pyrimidines, melibiose fermentation, sorbitol utilization, or degradation of pollutants. The sequences are deposited in EMBL under the accession numbers AL404849-AL407376.

6. A. Lépingle, S. Casaregola, C. Neuvéglise, E. Bon, H-V. Nguyen, F. Artiguenave, P. Wincker, C. Gaillardin. Genomic exploration of the hemiascomycetous yeasts: *Debaryomyces hansenii* var. *hansenii.* FEBS Lett **487**:82-86.

By analyzing 2830 random sequence tags (RSTs), totalling 2.7 Mb, we explored the genome of the marine, osmo-and halotolerant yeast, *Debaryomyces hansenii*. A contig 29 kb in length harbors the entire mitochondrial genome. The genes encoding Cox1, Cox2, Cox3, Cob, Atp6, Atp8, Atp9, several subunits of the NADH dehydrogenase complex 1 and 11 tRNAs were unambiguously identified. An equivalent number of putative transposable elements compared to *Saccharomyces cerevisiae* were detected, the majority of which are more related to higher eukaryote *copia* elements. BLASTX comparisons of RSTs with databases revealed at least 1119 putative open reading frames with homology to *S. cerevisiae* and 49 to other genomes. Specific functions, including transport of metabolites, are clearly overrepresented in *D. hansenii* compared to *S. cerevisiae*, consistent with the observed difference in physiology of the two species. The sequences have been deposited with EMBL under the accession numbers AL436045-AL438874.

7. S. Casaregola, C. Neuvéglise, E. Bon, C. Feynerol, F. Artiguenave, P. Wincker, C. Gaillardin. Genomic exploration of the hemiascomycetous yeasts: *Yarrowia lipolytica*. FEBS Lett. **487**: 95-100.

A total of 4940 random sequence tags of the dimorphic yeast *Yarrowia lipolytica*, totalling 4.9 Mb, were analyzed. BLASTX comparisons revealed at least 1229 novel *Y. lipolytica* genes 1083 genes having homology with *Saccharomyces cerevisiae* genes and 146 with genes from various other genomes. This confirms the rapid sequence evolution assumed for *Y. lipolytica*. Functional analysis of newly discovered genes revealed that several enzymatic activities were increased compared to *S. cerevisiae*, in particular, transport activities, ion homeostasis, and various metabolism pathways. Most of the mitochondrial genes were identified in contigs spanning more than 47 kb. Matches to retrotransposons were observed, including a *S. cerevisiae* Ty3 and a LINE element. The sequences have been deposited with EMBL under the accession numbers AL409956-AL414895.

8. C. Gaillardin, G. Duchateau-Nguyen, F. Tekaial, B. Llorente, S. Casaregola, C. Toffano-Nioche, M. Aigle, F. Artiguenave, G. Blandin, M. Bolotin-Fukuhara, E. Bon, P. Brottiere, J. de Montigny, B. Dujon, P. Durrens, A. Lépingle, A. Malpertuy, C. Neuvéglise, O. Ozier-Kalogeropoulos, S. Potier, W. Saurine, M. Termier, M. Wésolowski-Louvel, P. Wincker, J-L Souciet,J. Weissenbach. 2000. Genomic exploration of the hemiascomycetous yeasts: comparative functional classification of genes. FEBS Lett. **487**:134-149.

We explored the biological diversity of hemiascomycetous yeasts using a set of 22 000 newly identified genes in 13 species through BLASTX searches. Genes without clear homologue in *Saccharomyces cerevisiae* appeared to be conserved in several species, suggesting that they were recently lost by *S. cerevisiae*. They often identified well-known species-specific

traits. Cases of gene acquisition through horizontal transfer appeared to occur very rarely if at all. All identified genes were ascribed to functional classes. Functional classes were differently represented among species. Species classification by functional clustering roughly paralleled rDNA phylogeny. Unequal distribution of rapidly evolving, ascomycete-specific, genes among species and functions was shown to contribute strongly to this clustering. A few cases of gene family amplification were documented, but no general correlation could be observed between functional differentiation of yeast species and variations of gene family sizes. Yeast biological diversity seems thus to result from limited species-specific gene losses or duplications, and for a large part from rapid evolution of genes and regulatory factors dedicated to specific functions.

XVIII. Research institute for Viticulture and Enology, Matuškova 25 833 11 Bratislava, Slovakia. Communicated bu E. Minárik.

The following papers were recently published or accepted for publication.

1. E. Minárik. 2000. Contamination of wines by yeasts in the course of production and ageing. Vinohrad **38** suppl. 7 (in Slovak).

The evolution of modern techniques in the control of barrel contamination was investigated. The application of effective methods of decontamination and the control of oak barrels during the process of wine production and ageing enables perfect elimination of contaminating yeasts and yeast-like microorganisms, limiting thus their unfavourable effect on cleanness, taste and typical varietal wine character. Microbiological and technological aspects are discussed.

2. E. Minárik.2O01, influence of spontaneous grape must fermentation on 2-phenylethanol content in wine. Vinohrad, accepted for publication (in Slovak).

Spontaneous alcoholic fermentation of grape must shows increased 2-phenylethanol and glycerol formation by yeasts compared with musts fermented by pure yeast starters. In some cases over 250 mg/l phenylethanol may be formed by the spontaneous yeast flora, but only 20-40 mg/l in wines fermented by selected yeast strains. As 2-phenylethanol participates in the bouquet formation, some winemakers believe that spontaneously fermented wines display a broader area for completing the character of the wine which might be a well founded old argumentation.

3. E. Minárik. 2001. Red wine ageing on fine lees. Vinič a víno, accepted for publication (in Slovak).

Recent studies confirm differences in releasing mannoproteins bp different yeast strains during, alcoholic fermentation influencing thus stability, body and persistence of red wine colour. The selection of yeast strain for wine ageing “sur lies" is very important from the point of view of releasing mannoproteins during fermentation and ageing (autolysis of dead yeast cells).

4. E. Minárik, 2001. Optimization of efficacy conditions of active dry wine yeasts. Vinič a víno, accepted for publication (in Slovak).

The following basic requirements are dealt with: a) producing conditions for the predominance of pure yeast starters over the spontaneous yeast flora, b) revitalization of selected dry yeast starters, c) suitability of the yeast strain for white grape must, red must and mash fermentation, d) analytical and sensory qualities of selected yeast strains.

5. E. Minárik. 2001. White wines fermented in the presence of oak wood cuttings (chips). Vinohrad, in press (in Slovak).

White wines fermented in the presence of oak cuttings show higher volatile substance production and better sensory scores compared with control wines. The complex wine aroma is considerably increased by vanillin, eugenol, and cis-lactones of the oak wood. American oak provided highest amount of cis-lactones. Wine yeasts metabolize vanillin and furfural resulting in vanillyl- und furfuryl alcohol. Application of oak wood cuttings enables fermentation in large steel vessels and tanks, by which loss of aromatic substances or colour change of the wine may be reduced.

XIX. Department of Biology, Faculty of Medicine, Masaryk University, 66243 Brno, Czech Republic. Communicated by M. Kopecká <mkopecka@med.muni.cz>.

Original papers (in extenso).

1. Kopecká M., Gabriel M., Takeo K., Yamaguchi M., Svoboda A., Ohkusu M., Hata K., Yoshida S. 2001. Microtubules and actin cytoskeleton in *Cryptococcus neoformans* compared with ascomycetous budding and fission yeasts. Eur. J. Cell Biol. **80**:303-311.

2. Slaninová, I., Šesták, S., Svoboda, A. ,Farkaš, V. 2001. Cell wall and cytoskeleton reorganization as the response to hyperosmotic shock in *Saccharomyces cerevisiae*. Arch. Microbiol. **17**:245-52.

Abstracts of lectures and posters.

3. Gabriel, M., Ishiguro, J., Kopecká, M., Svoboda, A. 2000. Study of cps mutants of *Schizosaccharomyces pombe.* Folia Microbiol. **45**:72.

4. Kopecká, M., Gabriel, M., Svoboda, A., Takeo, K., Yamaguchi, M., Ohkusu, M., Hata, K., Yoshida, S. 2000. Cytoskeleton and ultrastructure in some human fungal pathogens. Folia Microbiol. **45**:78.

5. Slaninová, I., Kucsera, J., Svoboda, A. 2000. Cytoskeleton and mitochondrial topology in *Xantophyllomyces dendrorhous (Phaffia rhodozyma).* Folia Microbiol. **45**:89.

6. Slaninová, I., Šesták, S., Svoboda, A., Farkaš, V. 2000. Osmotic shock affects cell wall and cytoskeleton in *Saccharomyces cerevisiae*. Cell. Mol. Biol. (Congress Vol.) **46**:231.

7. Svoboda,A. 2000. Pathway of secreted molecules through the cell. Cells II, Kopp nakladatelství, Èeské Budìjovice, pp. 61-63.

8. Ishiguro, J., Shimada,S., Gabriel, M.,Kopecká, M. 2000. Characterization of a fission yeast mutant which displays defects in cell wall integrity and cytokinesis. In: Tenth International Symposium on Yeasts: "The rising power of yeasts in science and industry" Papendal, Arnhem, The Netherlands 27 August – 1 September, 2000. J.P.van Dijken, W.A.Scheffers (eds). Delft University Press, The Netherlands, pp.173.

9. Gabriel, M., Ishiguro, J., Kopecká, M. 2000. Cytoskeleton and morphological aberrations of septum formation and cytokinesis in cps6 mutant of fission yeast, *Schizosaccharomyces pombe*. VIII. Cytoskeletální klub, Vranovská Ves 15.-17. 3. 2000, s.11.

8. Kopecká, M., Gabriel, M., Svoboda, A., Takeo, K., Yamaguchi, M., Ohkusu, M., Hata, K., Yoshida, S. 2000. Cytoskeleton in some pathogenic fungi. VIII. Cytoskeletální klub, Vranovská Ves 15.-17. 3. 2000, s.12.

Network: *Yeasts in Food and Beverages*: Publications regarding Yeasts in Food and Beverages

Coordinated by P. Romano <pot2930@iperbole.bologna.it>

Brazil: Dep. Bioquimica/lQ-UFRI, Av. Brig. Trompowysk, S/n - C.T. - Bloco A, Al 534-A-CEP 21949-900, Rio de Janeiro. Communicated by Elis Eleutherio <eliscael@iq.ufrj.br>.

1. Eleutherio, E.C., Araujo, P.S., Panek, A.D. 1993. Protective role of trehalose during heat stress in *Saccharomyces cerevisiae*. Cryobiology. **30**(6):591-6. PMID: 8306706; UI: 94139359.

Yeast cells accumulate the disaccharide trehalose in response to certain stress conditions. In an attempt to verify the role that trehalose plays when yeast cells are faced with heat stress, yeast mutant strains with specific lesions in trehalose metabolism were used. Cultures growing exponentially on glucose were shifted from 28 to 40 degrees C for 1 h. Accumulation of trehalose was correlated with heat tolerance, measured as resistance to 50.5 degrees C. Additionally, it was observed that the trehalose carrier was not involved in the mechanism of thermotolerance acquisition. Mutants that lack the carrier were also able to acquire thermotolerance as long as synthesis of the disaccharide took place.

2. Eleutherio, E.C., Araujo, P.S., Panek, A.D. 1993. Role of the trehalose carrier in dehydration resistance of *Saccharomyces cerevisiae*. Biochim Biophys Acta. **1156**(3):263-6. PMID: 8461315; UI: 93213829.

Yeast cells are well known for their ability to survive complete dehydration, a phenomenon that is directly linked to the presence of the sugar trehalose in these cells. This sugar apparently endows the cells with the capacity to survive dehydration. Previous studies on in vitro models showed that trehalose must be present on both sides of the bilayer to stabilize dry membranes. The present report demonstrates that a specific trehalose carrier seems to enable the sugar to protect the yeast cell membrane by translocating trehalose from the cytosol to the extracellular environment. Saccharomyces cerevisiae mutant strains which lack the trehalose carrier did not survive after dehydration although they accumulated endogenous trehalose. Furthermore, when carrier mutants were dehydrated in the presence of exogenous trehalose the cells became more resistant showing increased survival.

3. Eleutherio, E.C., Ribeiro, M.J., Pereira, M.D., Maia, F.M., Panek, A.D. 1995. Effect of trehalose during stress in a heat-shock resistant mutant of *Saccharomyces cerevisiae*. Biochem Mol Biol Int. **36**(6):1217-23. PMID: 8535293; UI: 96070127.

Cells of a heat-shock resistant mutant were approximately 1000-times more resistant to lethal heat shock than those of the parental strain. We observed that exponentially growing cells of the mutant synthesized trehalose and showed increased osmotolerance, dehydration tolerance an ethanol tolerance, a fact not observed in wild type strains. The mutant synthesizes constitutively six proteins, among them two proteins of 56 and 63 kDa. Interestingly these molecular weights could correspond to the subunit of trehalose-6-phosphate synthase and to phosphoglucomutase II, respectively. Our results showed that glucose-growing cells of the hsr 1 mutant possessed high levels of activity of these enzymes when compared to the control strain.

4. Cuber, R., Eleutherio, E.C., Pereira, M.D., Panek, A.D. 1997. The role of the trehalose transporter during germination. Biochim Biophys Acta. **1330**(2):165-71. PMID: 9408169; UI: 98070107.

Previous studies on the resistance of yeast cells to dehydration pointed towards the protective role of trehalose and the importance of the specific trehalose transporter in guaranteeing survival. The present report demonstrates that the trehalose transporter is essential during the germination process in order to translocate trehalose from the cytosol to the external environment. Diploids that lack the trehalose transporter germinate poorly and do not form 4 spore tetrads although they accumulate trehalose and show trehalase activity. Furthermore, addition of exogenous trehalose to the germination medium enhances germination and normal segregation. The ability to transport trehalose is dominant and seems to be related to a single gene.

5. Eleutherio, E.C., Maia, F.M., Pereira, M.D., Degre, R., Cameron, D., Panek, A.D. 1997. Induction of desiccation tolerance by osmotic treatment in *Saccharomyces uvarum* var. *carlsbergensis*. Can J Microbiol. **43**(5):495-8. PMID: 9165704; UI: 97308478.

Saccharomyces uvarum var. *carlsbergensis* is heat sensitive and when dried by usual procedures exhibits very poor survival. Our results demonstrate that these cells are capable of accumulating trehalose when submitted to an osmotic treatment using 20% solutions of either sorbitol or dextrin endowing them with the capacity of surviving posterior dehydration.

6. Eleutherio, E.C., Silva, J.T., Panek, A.D. 1998. Identification of an integral membrane 80 kDa protein of *Saccharomyces cerevisiae* induced in response to dehydration. Cell Stress Chaperones **3**(1):37-43. PMID: 9585180; UI: 98244579.

Using SDS-PAGE gels we observed the induced synthesis of a protein with a molecular mass of 80 kDa when cells of strains of *Saccharomyces cerevisiae* were subjected to dehydration. Physiological analysis showed that this protein is not present during growth on glucose but was found in derepressed cells from stationary phase. Furthermore, its synthesis was induced when cells were grown on medium containing alpha-methyl-glucoside as carbon source. However, the 80 kDa protein was not found in cells of mutants unable to transport trehalose. This protein was localized in the cytoplasmic membrane and showed trehalose-binding activity, determined by its partial purification on a trehalose-Sepharose 6B affinity column. The possible involvement of the 80 kDa protein with the trehalose transport system is discussed.

France: Laboratoire de Genetique Moleculaire, GM. Bat. 400, 91405 Orsay Cedex. Communicated by Monique Bolotin-Fukuhara <igmors.u-psud.fr>.

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1. Billard, P, Dumond, H, Bolotin-Fukuhara, M. 1997. Characterization of an AP1-like transcription factor that mediates oxidative stress response in *Kluyveromyces lactis*. Mol. Gen. Genet. **257**:62-70.

2. Bourgarel, D, Nguyen, C-C, Bolotin-Fukuhara, M. 1999. HAP4, the glucose-repressed regulated subunit of the AP transcriptional complex involved in the fermentation-respiration shift has a functional homolog in the respiratory yeast *Kluyveromyces lactis*. Mol. Microbiol. **31**:1205-1216.

3. Bolotin-Fukuhara, M., Š., Wésolowski-Louvel, M. 2000. Genomic Exploration of the Hemiascomycetous Yeasts: 11. *Kluyveromyces lactis* FEBS Lett. **487**:66-70.

4. Dumond, H, Danielou, N, Pinto, M, Bolotin-Fukuhara, M. 2000. Large-scale study of Yap1p-dependent genes in standard and H_2O_2 stress conditions: role of Yap1p in cell proliferation control in yeast. Molec. Microbiol. **36**:830-845.

France: Université Henri Poincaré, Nancy 1, UMR UHP-CNRS 7564 – LCPE, Faculté de Pharmacie, Laboratoire de Biochimie Microbienne 5, rue Albert Lebrun B.P. 403, 54001 NANCY Cedex. Communicated by Joël Coulon.

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1. Coulon, J., Bonaly, R., Pucci, B., Polidori, A., Barthelemy, P., Contino, C. 1998. Cell targeting by glycosidic telomers. Specific recognition of the Kb CWL1 lectin by galactosylated telomers. Bioconjugate Chemistry **9**(2):152-159.

This work deals with the synthesis and lectinic recognition ability of galactosylated telomers. To investigate if telomeric carriers could exhibit cellular recognition properties, we have synthesized mono- and polygalactosylated tris(hydroxymethyl)acrylamidomethane (THAM) telomers. The affinity of such macromolecular drug carriers toward a receptor, the yeast Kb CWL1 lectin, was defined, and the influence of mono- or polygalactosylation of THAM units on the recognition phenomenon was assessed. The lectinic affinity of the compounds was estimated by measuring the inhibition of yeast aggregation. The average degree of polymerization as well as the hydrophilic-lipophilic balance of such galactosylated telomers affects their recognition ability for the lectin.

2. Rakotoarivony Iung, A., Coulon, J., Ferenc, K., Ngondi-Ekomé, J., Vallner, J., Bonaly, R. 1999. Mitochondrial function in cell wall glycoprotein synthesis in *Saccharomyces cerevisiae* NCYC 625 (wild type) and [*rho*°] mutants. Appl. Environ. Microbiol. **65**(12):5398-5402.

We studied phosphopeptidomannans (PPMs) of two *Saccharomyces cerevisiae* NCYC 625 strains (*S. diastaticus*): a wild type strain grown aerobically, anaerobically, and in the presence of antimycin and a [*rho*°] mutant grown aerobically and anaerobically. The aerobic wild-type cultures were highly flocculent, but all others were weakly flocculent. Ligands implicated in flocculation of mutants or antimycin-treated cells were not aggregated as much by concanavalin A as were those of the wild type. The [*rho*°] mutants and antimycin-treated cells differ from the wild type in PPM composition and invertase, acid phosphatase and glucoamylase activities. PPMs extracted from different cells differ in the protein but not in the glycosidic moiety. The PPMs were less stable in mitochondrion-deficient cells than in wild-type cells grown aerobically, and this difference may be attributable to defective mitochondrial function during cell wall synthesis. The reduced flocculation of cells grown in the presence of antimycin, under anaerobiosis, or carrying a [*rho*°] mutation may be the consequence of alterations of PPM structures which are the ligands of lectins, both involved in this cell-cell recognition phenomenon. These respiratory chain alterations also affect peripheral, biologically active glycoproteins such as extracellular enzymes and peripheral PPMs.

3. El-Behhari, Géhin, G., Coulon, J., Bonaly, R. 2000. Evidence for a lectin in *Kluyveromyces* sp. that is involved in co-flocculation with *Schizosaccharomycs pombe.* FEMS Microbiol. Lett. **184**:41-46.

Co-flocculation is the aggregation of yeasts belonging to different genera or species. *Kluyveromyces bulgaricus* and *Kluyveromyces lactis* 5c are self-flocculent, but they can also co-flocculate with the non-flocculent yeast *Schizosaccharomyces pombe* 972 h^-. This co-flocculation is inhibited by D-galactose and galactose derivatives and involves the binding of a galactose-specific proteinic receptor (or lectin) of *Kluyveromyces sp.* to the cell wall galactomannans of *S. pombe*. The proteinic receptor is strongly anchored in the cell wall, it was partially purified by affinity chromatography using immobilized *S. pombe* galactomannans. This galactose-specific proteinic receptor does not appear to interfere in *K. bulgaricus* or *K. lactis* self-flocculation, which is mediated by another galactose-specific lectin weakly linked at the cell wall.

4. Coulon, J., Thiébault, F., Contino, C., Polidori, A., Bonaly, R., Pucci, B. 2000. Permeability of yeast cell envelope to fluorescent galactosylated telomers derived from THAM. Bioconjugate Chemistry **11**(4):461-468.

5. Géhin, G., Bonaly, R., Coulon, J. 2001. Isolation and biochemical characterization of cell wall tight complex involved in self-flocculation of *Kluyveromyces bulgaricus.* Antonie van Leeuwenhoek: in press.

Flocculation of yeasts is a cell-cell aggregation phenomenon which is driven by interactions between cell wall lectins and cell wall heteropolysaccharides. In Sabouraud medium, *Kluyveromyces bulgaricus* was highly flocculent. Incubation of flocculent *K. bulgaricus* cells with EDTA or Hecameg® led to extracts showing hemagglutinating and flocculating properties. Purification of the extracts by native PAGE gave two bands which allowed flocculation of deflocculated *K. bulgaricus*. Both bands with specific reflocculating activity were composed of five subunits whom only 3 possessed weak reflocculating activity upon deflocculated activity. The mixture of these three proteins allow to recover initial specific reflocculating activity of the complex. These three proteins denoted p28, p36 and p48 presented in their 15-first aminoacids homologies with glycolysis enzymes, i.e. respectively 3-phosphoglycerate mutase, glyceraldehyde-3-phosphate dehydrogenase and enolase. However no such enzymatic activity could be detected in the crude extract issued from treatment with EDTA and Hecameg® of flocculent yeast cells. When yeasts had grown in glucose poor medium, flocculation was drastically affected. The EDTA and Hecameg® crude extracts showed weak reflocculating activity. After PAGE, the protein complexes did not appear in the EDTA extract, but they did appear in the Hecameg® crude extract. These results suggest that : (i) self-flocculation of *K. bulgaricus* depends on the expression of different floc-forming protein complex, (ii) these proteins are galactose specific lectins showing homologies in their primary structure with glycolysis enzymes.

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1. Nguyen, H.-V., Pulvirenti, A. , Gaillardin, C. 2000. Rapid differentiation of the closely related *Kluyveromyces lactis* var. *lactis* and *K. marxianus* strains isolated from dairy products using selective media and PCR/RFLP of the rDNA non transcribed spacer 2. Can J. Microbiol. **46**: 1115-1122.

PCR/RFLP of the NTS2 (IGS2) of rDNA was applied to differentiate two losely related species *Kluyveromyces lactis* var. *lactis* (referred to as *K. lactis*) and *K. marxianus*. Using specific primers, he NTS2 region was amplified from DNA of both *K. lactis* and *K. marxianus* type and collection trains. AluI restriction of amplified fragments generated patterns characteristic for each species. PCR/RFLP of the NTS2 appears to be a convenient method for rapid identification of *K. lactis* and *K. marxianus*, frequently found in dairy products. Especially yeast strains collected from whey or cheese samples and scoring blue on X-gal glucose plates were either *K. lactis* or *K. marxianus*. An approach for quickly screening for *K. lactis/marxianus* and *Saccharomyces cerevisiae* in dairy products using X-gal coloured and lysine growth media was proposed for application purposes.

2. Corredor, M., Davila, A.-M., Gaillardin, C. , Casaregola, S. 2000. DNA probes specific for the yeast species related *Debaryomyces hansenii*: useful tools for rapid identification. FEMS Microbiol. Lett. **193**:171-177.

We developed a rapid and sensitive identification method for the halotolerant yeast *Debaryomyces hansenii*, based on the hybridization of species-specific sequences. These sequences were first identified in a survey of *D. hansenii* strains by random amplification of polymorphic DNA (RAPD) as giving conserved bands in all isolates tested. Two such conserved RAPD products, termed F0lpro and M18pro, were cloned from the type strain CBS 767. The specificity of these probes was assessed by hybridizing them to DNA from various species of yeasts commonly found in cheese. F0lpro and M18pro hybridized to the DNA of all *D. hansenii* var. hansenii tested, but not to DNA of other yeast species including the closely related strain of *D. hansenii* var. fabryii CBS 789. Hybridization patterns of F0lpro and M18pro on digested genomic DNA of *D. hansenii* indicated that the sequences were repeated in the genome of all *D. hansenii* var. *hansenii* tested, and gave distinct polymorphic patterns. The single F0lpro probe generated 11 different profiles for 24 strains by restriction fragment length polymorphism, using one restriction enzyme. F0lpro represents a new type of repeated element found in fungi, useful for both identification and typing of *D. hansenii* and, together with M18pro, simplifies the study of this species in complex flora.

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1. Dequin, S., Baptista, E., Barre, P. 1999. Acidification of grape musts by *S. cerevisiae* wine yeast strains genetically engineered to produce lactate. Am. J. Enol. Vit. **50**: 45-50.

Engineered *Saccharomyces cerevisiae* strains overexpressing a bacterial lacticodehydrogenase (LDH) have been described to perform mixed lactic acid-alcoholic fermentation under laboratory conditions (7). The acidification properties of these strains might be of great value for fermented beverages and especially in enology to solve problems of insufficient acidity in hot regions. To

assess the relevance of this model during alcoholic fermentation under enological conditions, eight wine strains expressing the LDH gene on a multicopy plasmid were constructed. The level of L(+) lactic acid produced and the resulting acidification were shown to be influenced by the strain and to a greater extent by the grape must composition. Wines obtained by lactic acid-alcoholic fermentation of seven grape musts contained 2.6 to 8.6 g/L of L(+) lactic acid. In comparison with classic alcoholic fermentation, lactic acid-alcoholic fermentation resulted in a decrease of 0.2 to 0.35 pH units in wines containing 5 to 8 g/L of lactic acid. Two grape musts with moderate or low acidity levels (pH 3.36 and pH 3.75) were acidified effectively (50% increase in total acidity, 0.17 and 0.27 pH unit decrease) by the production of 5 g/L of L(+) lactic acid by an engineered wine yeast strain. A reduction of 0.25 percent (vol/vol) of alcohol was also observed as a result of the diversion of carbon towards L(+) lactic acid. The volatile acidity was unchanged in the wine obtained with the engineered strain compared to the control strain. Despite a slower fermentation rate, the ability to ferment grape musts and the growth characteristics were not affected.

2. Fornairon, C., Mazauric, J.P., Salmon, J.M., Moutounet, M. 1999. Evidence for an oxygen consumption by *Saccharomyces cerevisiae* during maturation of wines on their lees. Journal International des Sciences de la Vigne et du Vin, **33** (2):79-86.

Traditional enological practices (« Bâtonnage » or « microoxygénation » techniques) during wine aging on yeast lees include limited repetitive additions of small amounts of oxygen to the wines. Such empirical practices are generally associated with a limited homogenisation of wine and lees. In this study, the potential relationship between oxygen consumption and the presence of wine lees during wine aging was investigated. Strong oxygen uptake rates by yeast lees were observed during wine aging at 16°C on total yeast lees obtained after fermentation of either synthetic medium or red and white grape musts. These specific oxygen utilization rates by yeast lees is always comprised between 3 and 11 $\mu g\, O_2\, h^{-1}\, 10^{-9}$ cells from the second to the sixth month of aging. The initial levels of specific oxygen utilization rates and the time-decay of these rates along wine aging were very dependent on yeast strains. However such oxygen utilization rates by yeast lees could be responsible for the total dissolved oxygen depletion from wines in less than 20 hours at 16°C during aging on total lees. Such results were of particular importance to evaluate the exact timing of oxygen additions during wine aging on lees. Further experiments had to be done to determine the biological or chemical nature of such oxygen consumption by lees. Such oxygen consumption by yeast lees may lead to final reaction products which may exert strong organoleptic effects on the final quality of wines.

3. Rachidi, N., Barre, P., Blondin, B. 1999. Multiple Ty mediated chromosomal translocations lead to karyotype changes in a *Saccharomyces cerevisiae* wine yeast. Mol. Gen. Gen. **261**:841-850.

Enological *S. cerevisiae* yeasts display high chromosome length polymorphism but the molecular bases of this phenomenon have not yet been clearly defined. In order to gain further insight into the molecular mechanisms responsible for the karyotype alterations, we examined the chromosomal constitution of a strain shown to possess aberrant chromosomes. Our data revealed that the analyzed strain was bearing four rearranged chromosomes resulting from two reciprocal translocations between chromosomes III and I, and chromsomes III and VII. The size of the chromosomal fragments exchanged through the translocations ranged from 40 to 150 kb. Characterization of the breakpoints indicated that the translocations involved the RAHS of chromosome III, a transposition hot-spot on the right arm of chromosome I and a region of the left arm of chromosome VII. An analysis of the junctions showed that Ty were present on all of them and suggested that the translocations result from recombination between Ty transposable elements. The evidence of multiple translocations through Ty elements in a single strain suggests that spontaneous Ty driven rearrangement could be quite common and play a major role in the alteration of karyotypes in natural or industrial yeasts.

4. Remize, F., Roustan, J.L., Sablayrolles, J.M., Barre, P., Dequin, S. 1999. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. Appl. Environ. Microbiol. **65**: 143-149.

Six commercial wine yeast strains and three non-industrial strains (two laboratory strains and one haploid derived from a wine yeast strain) were engineered to produce high amounts of glycerol with lower ethanol yield. Overexpression of the *GPD1* gene encoding a glycerol-3-phosphate dehydrogenase resulted in a 1.5- to 2.5-fold increase in glycerol production and a slight decrease in ethanol formation under conditions simulating wine fermentation. All the strains overexpressing *GPD1* produced a higher amount of succinate and acetate, with marked differences in the level of these compounds between industrial and non-industrial engineered strains. Acetoin and 2,3-butanediol formation was enhanced with significant variation between strains and with the level of glycerol produced. Wine strains overproducing glycerol at moderate levels (12 to 18 g/l) almost completely reduced acetoin to 2,3-butanediol. A lower biomass concentration was attained by *GPD1* overexpressing strains, probably due to high acetaldehyde production during the growth phase. Despite the reduction in cell number, complete sugar exhaustion was achieved during fermentation in sugar-rich medium. Surprisingly, the engineered wine yeast strains exhibited a significant increase in the fermentation rate in stationary phase, which reduced the time of fermentation.

5. Grenier, P., Alvarez, I., Roger, J.M., Steinmetz, V., Barre, P., Sablayrolles, J.M. 2000. Artificial intelligence in wine-making. J. Int. Sci. Vigne vin, **34**:61-66.

In this paper, some terms of artificial intelligence are defined. Some present and potential applications of knowledge based systems are presented in the field of wine-making. Areas of concern were: multi sensor fusion, prediction by model cooperation, and diagnosis. Artificial intelligence techniques can indeed be applied for aiding the wine-maker in his choices. They facilitate the combination between experience and recent progress in technology. When associated with statistical processing, they allow knowledge sources to be used more effectively. Beyond wine-making, the prospects of artificial intelligence are promising for research and food industry, especially for improving the robustness of measuring systems (multi-sensors, sensors interpreted or validated by models) and for process diagnosis (risk prediction, action proposal).

6. Julien, A., Roustan, J.L., Dulau, L., Sablayrolles, J.M. 2000. Comparison of nitrogen and oxygen demands of enological yeasts: Technological consequences. Am. J. Enol. Vitic. **51**:215-222.

Nitrogen and oxygen requirements of commercial yeasts were compared. Nitrogen demands were measured by calculating the amounts of ammoniacal nitrogen necessary to keep the fermentation rate constant during the stationary phase. Oxygen demands were studied by (i) running anaerobic fermentations and (ii) measuring the effect of adding 1 mg/L oxygen at the beginning of the stationary phase. Substantial differences were found and their technological impact was discussed. Because of the importance of nitrogen and oxygen in enology, using such tests may be of great interest, especially when there are risks of (i) slow fermentations, due to nitrogen deficiencies or (ii) sluggish fermentations, mainly due to oxygen limitations.

7. Rachidi, N., Martinez, M.J., Barre, P., Blondin, B. 2000. *Saccharomyces cerevisiae* PAU genes are induced by anaerobiosis. Molecular Microbiol. **35:**1421-1430.

Saccharomyces cerevisiae PAU genes constitute the largest multigene family in yeast, with 23 members located mainly in subtelomeric regions. The role and regulation of these genes was previously unknown. We detected PAU gene expression during alcoholic fermentation. An analysis of PAU gene regulation using PAU-lacZ fusions and Northern analyses revealed that they were regulated by anaerobiosis. PAU genes display, however, different abilities to be induced by anaerobiosis and this appears to be related to their chromosomal localization: two subtelomeric copies are more weakly inducible than an interstitial one. We show that PAU genes are negatively regulated by oxygen and repressed by heme. Examination of PAU gene expression in rox1? and tup1? strains indicates that PAU repression by oxygen is mediated by an unknown, heme-dependent pathway, which does not involve the Rox1p anaerobic repressor but requires Tup1p. Given the size of the gene family, PAU genes could be expected to be important during yeast life and some of them probably help the yeast to cope with anaerobiosis.

8. Rachidi, N., Barre, P., Blondin, B. 2000. Examination of the transcriptional specificity of an enological yeast. A pilot experiment on the chromosome-III right arm. Curr Genet. **37**(1):1-11.

The adaptation of yeasts to industrial environments is thought to be largely dependent on gene expression specificity. To assess the transcriptional specificity of an enological strain, we performed a pilot experiment and examined the transcript level of 99 ORFs of the chromosome III right arm with two strains, an enological-derived strain and a laboratory strain, grown under different physiological conditions : respiration, standard alcoholic fermentation and enological alcoholic fermentation. The use of 99 single ORF-derived probes led to the detection of 49 transcripts, most of which were present at low levels and not regulated. Ethanol respiration induced transcripts in a similar manner with both strains. While standard alcoholic fermentation led to only minor regulations, the enological fermentation conditions triggered a transcriptional response in the enological-derived strain alone. The known or predicted functions of several genes induced under enological conditions is related to alcoholic fermentation or stress, suggesting that their specific induction could reflect adaptation of the strain to the enological environment. Our data suggest that systematic transcriptional studies are an effective way to assess the molecular basis of yeast adaptation to industrial environments.

9. Remize, F., Sablayrolles, J.M., Dequin, S. 2000. Re-assessement of the influence of yeast strain and environmental factors on glycerol production in wine. J. Appl. Microbiol. **88**:371-378.

Increasing glycerol production is a matter of concern for wine makers to improve the quality of certain wines. We have compared the impact of strain and relevant environmental factors influencing glycerol production under the same conditions i.e. standardized conditions simulating enological fermentation. Glycerol production of 19 industrial wine strains ranged from 6.4 to 8.9 g l-1 and varied significantly between strains. The production of acetate and succinate was also found to differ substantially depending on the strain but no significant strain-dependent variation was observed for acetaldehyde. Interestingly, high glycerol production was not correlated to high production of acetate or acetaldehyde that are undesirable in wine. A detailed study with two low- or two high-glycerol producers strains showed that temperature and the initial concentration of nitrogen had little effect on the amount of glycerol formed, although agitation or a nitrogen source composed mainly of ammoniacal nitrogen slightly enhanced glycerol production. The influence of environmental factors remained minor while the predominant factor for glycerol variability in wine was attributed to the strain. Taking into account winemaking constraints, the results indicate that achieving high glycerol content in wine requires the selection or improvement of yeast strains rather than the control of growth and cultivation conditions.

10. Remize, F., Andrieu, E. Dequin, S. 2000. Engineering of the pyruvate dehydrogenase by-pass in *S. cerevisiae*- Role of the cytosolic Mg2+ and mitochondrial K+ acetaldehyde dehydrogenases Ald6p and Ald4p in acetate formation during alcoholic fermentation.. Appl. Environ. Microbiol. **66**:3151-3159.

Acetic acid plays a crucial role in the organoleptic balance of many fermented products. We have investigated the factors controlling the production of acetate by *S. cerevisiae* during alcoholic fermentation by metabolic engineering of the enzymatic steps involved in its formation and its utilisation. The impact of reduced pyruvate decarboxylase (PDC), limited acetaldehyde dehydrogenase (ACDH), or increased acetoacetyl CoA synthetase (ACS) levels in a strain derived from a wine yeast strain was studied during alcoholic fermentation. In the strain deleted for the *PDC1* gene exhibiting 25% of PDC activity of the wild type, no significant differences were observed in the acetate yield or in the amount of secondary metabolites formed. A strain overexpressing *ACS2* and displaying a 4- to 7-fold increase in acetoacetyl CoA synthetase activity did not produce reduced acetate levels. In contrast, strains disrupted for one or two copies of *ALD6* encoding the cytosolic Mg2+-activated NADP-dependent Acdhp (exhibiting 60 and 30% of wild type ACDH activity) showed a substantial decrease in acetate yield (the acetate production was 75 and 40% of wild-type respectively). This decrease was associated with a rerouting of carbon flux towards the formation of glycerol, succinate and butanediol. The deletion of *ALD4* encoding the mitochondrial K+-activated NAD(P)-linked ACDH had no effect on the amount of acetate formed. In contrast, a strain lacking both Ald6p and Ald4p exhibited a long delay in growth and acetate production, suggesting that Ald4p can partially replace Ald6p isoform. Moreover, the *ald6 ald4* double mutant was still able to ferment high sugar amounts and to produce acetate, suggesting the contribution of other member(s) of the *ALD* family.

11. Salmon, J.M., Fornairon-Bonnefond, C., Mazauric, J.P., Moutounet, M.. 2000. Oxygen consumption by wine lees : impact on lees integrity during wine ageing. Food Chem., **71** (4):519-528.

During wine ageing on lees, periodic stirring of the lees and repetitive additions of small amounts of oxygen to the wines is generally performed on a traditional and empirical basis. Although this technological step is thought to have a significant impact on the organoleptic composition of the final product, few reports dealt with the effect of the interaction between oxygen and lees during such a process. During simulation of wine ageing on lees, we demonstrated that some membrane lipids of yeast lees in contact with dissolved oxygen, even at very low concentration, were submitted to a mild oxidation. These oxidation reactions led first to the production of lipid peroxides and then to the production of unknown end products, which were suspected to be kept within the membranes of lees. These oxidation reactions were primarily due to the production of reactive oxygen species (ROS), and totally explained the capacity of yeast lees to consume oxygen during wine ageing. The lipid oxidations within the plasma membrane were responsible for strong modifications of plasma membrane order. One can expect that some of the final products of oxidation reactions may play a favourable role in the organoleptic equilibrium of wines aged on lees.

12. Vila, I., Sablayrolles, J.M., Gerland, C., Baumes, R., Bayonove, C., Barre, P. 2000. Comparison of 'aromatic' and 'neutral' yeast strains: Influence of vinification conditions. Vitic. Enol. Sci. **55**:59-66.

The influence of two yeast strains, an 'aromatic' and a 'neutral' strain, on fermentation wine aroma was investigated. The influence of fermentation conditions (grape cultivar and fermentation scale) and of wine aging on aroma and volatile fermentation compounds was studied using both sensory and chemical analyses. The 3 factors tested had significant effect on wine aroma. Wines produced by the two strains were not always sensorial or chemially discriminated depending on fermentation condition on wine aging. These results show that the concept of 'aromatic' strain depends on vinification conditions. In some cases a relation between sensory and chemical analyses was pointed out; compounds detected by GC-olfactometry were at least partially responsible for differences observed between strains.

13. Alexandre, H., Ansanay-Galeote, V., Dequi, S., Blondin, B. 2001. Global gene expression during short term ethanol stress in *Saccharomyces cerevisiae*. FEBS Letters, *in press*.

DNA microarrays were used to investigate the expression profile of yeast genes in response to ethanol. Up to 3.1% of the genes encoded in the yeast genome were up-regulated by at least a factor of three after 30 minutes ethanol stress (7% v/v). Concomitantly, 3.2 % of the genes were down-regulated by a factor of three. Of the genes up-regulated in response to ethanol 49.4% belong to the ESR (Environmental Stress Response) and 14.2% belong to stress gene family. Our data show that in addition to the previously identified ethanol-induced genes, a very large number of genes involved in ionic homeostasis, heat protection, trehalose synthesis and antioxidant defence also respond to ethanol stress. It appears that a large number of the up-regulated genes are involved in energy metabolism. Thus, "management" of the energy pool (especially ATP) seems to constitute an ethanol stress response and to involve different mechanisms.

14. Ansanay-Galeote, V., Blondin, B., Dequin, S., Sablayrolles, J.M. 2001. Stress effect of ethanol on fermentation kinetics by stationnary-phase cells of *Saccharomyces cerevisiae*. Biotechnology Letters, *in press*.

Alcoholic fermentation in winemaking conditions occurs mainly with yeasts in stationary phase. During this phase, the fermentation rate decreases progressively as the fermentation progresses. The factor which govern this decrease in fermentation rate are not well defined. In this study, we observed that ethanol plays a major role in the control of the fermentation rate. When simulating a natural ethanol production by a progressive addition of 4% (v/v) of ethanol on two strains exhibiting different fermentative abilities, K1 (a commercial wine strain) and V5 (a strain derived of a wine yeast), a direct correlation was observed between the rate of fermentation and the concentration of ethanol in the medium, with theses two strains. In contrast, the effect of sudden addition of 2%, 4% or 6% (v/v) of ethanol was different depending on the strain. While the same effect was observed for K1 whatever the way of ethanol addition, V5 required an adaptation period after the shock addition of ethanol. However, the ethanol shock seems to render the yeast cells less sensitive to a further increase in ethanol concentration. Theses observations point to a possible mechanism of cellular adaptation to ethanol stress. Moreover, the experimental model used here could be an excellent system to assess the gene response specific to ethanol stress under enological conditions.

15. Blateyron, L., Sablayrolles, J.M. 2001. Stuck and slow fermentations in enology: Statistical study of causes and effectiveness of combined additions of oxygen and diammonium phosphate. J. Biosc. Bioeng., *in press*.

One hundred and seventy-eight musts from different regions in France were selected by enologists and fermented under standardized conditions. Two kinds of fermentation problems were distinguished: (a) slow and (b) sluggish (late-onset sluggish) and stuck (with residual sugar) fermentations. Slow fermentations, characterized by a low fermentation rate throughout the process, were always due to low assimilable nitrogen concentrations in the must. The advantages of using formol titration for measuring assimilable nitrogen are discussed. In contrast, sluggish and stuck fermentations, characterized by very low yeast viability at the end of fermentation, could not be predicted from the analytical data even though their probability was increased when the initial sugar concentration was high. All problems of stuck and sluggish fermentations (concerning 40% of the musts) were solved by supplying 7 mg/l oxygen and 300 mg/l diammonium phosphate at the halfway of the fermentation process, which confirmed (i) the importance of these two nutrients in enological practices and (ii) the importance of adding them at the right time.

16. Camarasa, C., Bidard, F., Bony, M., Dequin, S., Barre, P. 2001. Characterization of *Schizosaccharomyces pombe* malate permease by expression in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol, *in press.*

In *Saccharomyces cerevisiae*, L-malic acid transport is not carrier-mediated and is limited to slow, simple diffusion of the undissociated acid. Expression in *S. cerevisiae* of the *MAE1* gene, encoding *Schizosaccharomyces pombe* malate permease, markedly increased L-malic acid uptake in this yeast. In this strain, at pH 3.5 (encountered in industrial processes), L-malic acid uptake involves *MAE1*-mediated transport of the monoanionic form of the acid (apparent kinetic parameters: Vmax = 8.7 nmol/mg/min; Km = 1.6 mM), and some simple diffusion of the undissociated L-malic acid (kD = 0.057 min-1). As total L-malic acid transport involved only low levels of diffusion, the *MAE1* permease was further characterized in the recombinant strain. L-malic acid transport was reversible and accumulative and depended on both the transmembrane gradient of the monoanionic acid form and on the ΔpH component of the protonmotive force. Dicarboxylic acids with stearic occupation closely related to L-malic acid, such as maleic, oxaloacetic, malonic succinic and fumaric acids, inhibited L- malic acid uptake, suggesting that these compounds use the same carrier. We found that increasing external pH directly inhibited malate uptake, resulting in a lower initial rate of uptake and a lower level of substrate accumulation. In *S. pombe*, proton movements, as shown by the internal acidification, accompanied malate uptake, consistent with the proton/dicarboxylate mechanism previously proposed. Surprisingly, no proton fluxes were observed during *MAE1*-mediated L-malic acid import in *S. cerevisiae* and intracellular pH remained constant. This suggests that, in *S. cerevisiae*, either there is a proton counterflow, or the *MAE1* permease functions differently from a proton/dicarboxylate symport.

17. Dequin, S. 2001. The potential of genetic engineering for improving brewing, wine-making and baking yeasts. Appl. Microbiol. Biotechnol., i*n press.*

The end of the 20th Century was marked by major advances in life technology, in particular areas related to genetics and more recently genomics. Considerable progress was made in the development of genetically improved yeast strains for the wine, brewing and baking industries. In the last decade, recombinant DNA technology widened the possibilities for introducing new properties. The most remarkable advances, which are discussed in this review, are improved process performance, off-flavor elimination, increased formation of by-products, improved hygienic properties or extension of substrate utilization. Although the introduction of this technology into traditional industries is currently limited by public perception, the number of potential applications of genetically modified industrial yeast is likely to increase in the coming years as our knowledge derived from genomic analyses increases.

18. Fornairon-Bonnefond, C., Camarasa, C., Moutounet, M., Salmon,J.M. 2001. New trends on yeast autolysis and wine ageing on lees : a bibliographic review. Journal International des Sciences de la Vigne et du Vin, **35** (2), *in press*.

In enology, lees are mainly used in the traditional practice of "sur lies" wine ageing, which consists of carrying on the contact between wine and lees (yeasts and vegetal residues) during ageing. Lees come either from first or second fermentation, and could be used for white or red wines elaboration. Such an enological practice remains yet empirical. In the present paper, the state of art was investigated in order to collect and analyze most of the scientific works realized on wine lees. It includes also technological points relevant from such a practice. A clear definition of wine lees from legal and technological points of view was given in the first part of the present paper. A second part described the mechanisms of autolysis and focused more precisely on each class of identified autolysis products. Many scientific works had indeed revealed the yeast autolysis phenomenon occuring during such a way of wine ageing. All these works remained mainly based on identification of yeast macromolecules released in the wine during the autolysis phenomenon. However, the experimental methodologies followed by the different authors are variable, and most of the obtained results were difficult to extrapolate to actual wine ageing on lees. Only few works dealt with the physicochemical properties of such lees during autolysis, specially towards oxygen, polyphenols and other wine compounds. A compilation of recent data obtained on these peculiar topics was then given. In a third and last part, the effect of ageing wine on lees was approached from a technical point of view.

19. Remize, F., Barnavon, L., Dequin, S. 2001. Glycerol export and glycerol 3-phosphate dehydrogenase, but not glycerol phosphatase, are rate limiting steps for glycerol production in *Saccharomyces cerevisiae*. Metabolic engineering, *in press*.

Glycerol, one of the most important by product of alcoholic fermentation, has positive effects on the sensory properties of fermented beverages. It has been recently shown that the most direct approach to increase glycerol formation is to overproduce the glycerol-3-phosphate dehydrogenase isoform Gpd1p. This study aimed at identifying other steps in glycerol synthesis or transport that may limit glycerol flux during glucose fermentation. We show that overproduction of Gpd2p, encoding the other isoform of glycerol-3-phosphate dehydrogenase is as effective as Gpd1p overproduction in increasing glycerol production (3.3-fold increase compared to the wild-type strain), and has similar effects on yeast metabolism. In contrast, overexpression of *GPP1* encoding glycerol 3-phosphatase did not enhance glycerol production. Moreover, strains overexpressing simultaneously Gpd1p and Gpp1p did not produce higher glycerol amounts than a strain overexpressing *GPD1*. These results demonstrate that the GPDH but not the phosphatase is rate-limiting for glycerol production. Glycerol export is mediated by the channel protein Fps1p. It has been recently shown that mutants lacking a region in the N-terminal domain of Fps1p constitutively release glycerol. We here show that cells expressing truncated Fps1p constructs during glucose fermentation compensate for glycerol loss by increasing glycerol production. Interestingly, the strain expressing a deregulated Fps1 glycerol channel had a different phenotype than the strain overexpressing *GPD* genes and showed poor growth during fermentation. Overexpression of *GPD1* in this strain even increased the amount of glycerol produced but lead to a much pronounced growth defect.

20. Salmon, J.M., Fornairon-Bonnefond, C., Mazauric, J.P., 2001. Interactions between wine lees and polyphenols : influence on oxygen consumption during simulation of wine aging. J. Food Sci., *in press*.

During wine ageing on lees, periodic stirring of the lees and repetitive additions of small amounts of oxygen to the wines is generally performed traditionally and empirically. During simulation of wine ageing on lees, it was recently demonstrated that some membrane lipids of yeast lees, in contact with dissolved oxygen at low concentration, undergo a mild oxidation explaining the capacity of yeast lees to consume oxygen during wine ageing. Since it is well known that tannins and pigments, already found in wine, may undergo oxidative reactions in the presence of oxygen, we studied in the present paper the crossed reactivity of wine polyphenols and yeast lees towards oxygen during simulation of wine ageing. We now show that the reactivity of polyphenols remaining in solution slightly increases as a function of contact time, while reactivity of yeast lees is strongly lowered, resulting in a whole decrease of reactivity towards oxygen by comparison with the reactivity of each component studied alone. A strong loss of reactivity of yeast lees towards oxygen is observed when separated from soluble polyphenols, although only a small fraction of the total polyphenols remained adsorbed on lees. Such effect may be related either with a strong collapsing of cytoplasmic yeast cell intermembrane space by some polyphenols, or a strong adhesion of polyphenols to the external part of the membrane cell surface, decreasing the accessibility and reactivity of oxygen reactive species towards membrane lipids.

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1. Alfenore, S., Delia, M.L., Strhaiano, P. 2000. Evaluation quantitative de l'effet killer des levures œnologiques. Effet de divers adjuvants.(Quantitative analysis of the killer activity of some enological yeasts. Effect of some additives.). J. Inter. Sci. Vigne Vin **34**(2): 41-47.

The Killer factor was discovered in 1963. Since this time it has been widely studied and nowadays a lot is known about genetics of the factor, the biochemistry of the toxin and also about the way of action of the toxin on sensitive yeasts. The yeast strains

are classified in three groups : killer strains, sensitive strains and neutral strains. The Killer strain is able to kill sensitive strains while neutral strains are unable to kill any strain but remain unaffected by the toxin. For sure this classification depends on the couple of strains (killer and sensitive) taken as references. It has been clearly established that the toxin acts on the sensitive cells by inducing important losses of ATP : due to holes created by the toxin in the cell membrane, ATP leaves the cell. n the field of Enology, it's generally accepted that a killer yeast has more probabilities to have a good implantation in a non sterile medium as the must is than a neutral or a sensitive strain. Nevertheless, it is difficult to have a precise idea of the sensitivity of a strain as well as of its toxicity, as most of the methods are only able to give a qualitative information. n this paper, a new method of evaluation of the killer effect is presented. Also some results dealing with its application to the classification of some enological yeast strains are given and discussed.. his method is based on the measurement of the ATP liberated by the sensitive strain under the action of the killer toxin. The criterion that we define is the initial rate of ATP losses, that means the quantity of ATP lost in the two first hours by the sensitive cells in contact with the toxin. In a first step, it is shown that this method is reliable and also that it is in a good agreement with the method using the flow cytometry. ATP leak could be correlated with the amount of affected cells (dead and damaged cells). So, using this method, it becomes possible to classifiy easily yeast strains in the respect of their sensitivity or toxicity. Several Killer yeast strains were tested against a sensitive stain and diferent sensitive strains were submitted to the action of a killer toxin produced by a killer strain.. It iwas shown that the losses of ATP were quite different according the sensitivity of the strain. The initial rate of ATP losses was found in a range of 0.00 to 0.12 micro mole/L/h. The second part of the work deals with the study of the possible effect of some products used for winemaking on the efficiency of the Killer effect. The products we have studied were bentonite, tanic acid and enological tanins. It is shown that these products may interact with the killer effect. So, bentonite, for example is able to inhibit completely the efficiency of the killer toxin, as soon as its concentration is about 10 grams/hL.

2. Aguilar Uscanga, M.G., Delia, M.L, Strehaiano, P. 2000. Nutritional requirements of *Brettanomyces bruxellensis*. Growth and physiology in batch and chemostat cultures. Can. J. Microbiol. **46**(11):1046-1050.

The nutritional requirements of *Brettanomyces bruxellensis* have been investigated. Batch culture and chemostat pulse techniques were used in order to identify growth-limiting nutrients. The study is organized in two parts: (1) the determination of essential components, (2) the quantification of medium components effect. Among the tested components, ammonium sulphate and yeast extract had a significant effect on glucose consumption, growth, and ethanol production. However, if the ammonium sulphate concentration is above 2 g/l, an inhibitory effect on *Brettanomyces bruxellensis* growth is observed. The yeast extract appears to be the most important and significant component for growth. The maximal amount of synthesized biomass is proportional to the concentration of yeast extract added to the culture broth (in the tested range). Magnesium and phosphate ions are probably not essential for *Brettanomyces bruxellensis*. It seems that these ions are supplied in sufficient amounts by the yeast extract in the culture medium. *Brettanomyces bruxellensis* strain appears to have very low nutritional requirements for growth.

3. Le Van, V.M., Strehaiano, P., N'Guyen, D.L., Taillandier, P. 2001. Microbial protease or yeast extract alternative additions for improvment of fermentation performance and quality of beer brewed with high rice content. J. Am. Soc. Brew. Chem. **59**(1):10-16.

Reduction of production costs is an important advantage of adjunct utilization in brewing. Nevertheless, 40% rice addition significantly decreases the biomass formation of yeast due to assimilable nitrogen depletion in wort. This leads to longer fermentation times and may have a negative influence on beer organoleptic quality. In this study, addition of protease to the malt-mash during mashing or supplementing yeast extract in wort after boiling in order to increase wort assimilable nitrogen content were compared. In both cases, the fermentation was considerably accelerated and the beer quality was significantly improved. Yeast extract gave better results than microbial protease. The fermentation kinetics and the beer quality from 60% malt–40% rice wort supplemented by yeast extract were the same as those from 100% malt wort.

4. Ramon Portugal, F., Da Silva, S., Strehaiano, P., Taillandier, P. 2001. New biotechnology answers to some old problems in winemaking. 11th World Congress of Food Science and Technology. Seoul. 22-27 April 2001.

The use of unusual yeasts or entrapped yeast cells may solve some problems in winemaking. For a lot of wines the alcoholic fermentation is followed by the malo-lactic fermentation due to specific lactic acid bacteria of which the control is not easy. The use of yeast *Schizosaccharomyces pombe* may be a possible solution. For sparkling wines, a technical problem is the removing of yeast cells after the in-bottle fermentation : a possible solution is the use of entrapped cells (*Saccharomyces*). Entrapped cells were prepared in sodium alginate : the inner layer contained the cells while the external layer was of sterile alginate. So, the cell escape in the medium is avoided. We showed that the malate degradation by *Schizosaccharomyces* was neither linked to growth nor to sugar assimilation and that it was possible to stop the activity of *Schizosacharomyces* by removing the beads. Many experiments were performed at a plant scale by using natural musts. The process is now currently used. For the sparkling wines, entrapped yeasts (*Saccharomyces*) were put in the bottle. It was shown that the fermentation kinetics as well as the organoleptic qualities were the

same as using free cells. But the removing of beads was greatly easier and quicker than this of free cells. After lab-scale experiments the process is now runing at the industrial level (Proenol SA). These examples show that some problems of traditional processes such as winemaking may be solved using unusual microorganism or a new way of use of classical microorganism.

5. Phowchinda, O., Delia, M. L., Strhaiano, P. 2001. *Brettanomyces* as a polluting yeast in alcoholic fermentation. 11th World Congress of Food Science and Technology. Seoul. 22-27 April 2001.

For the ten last years, *Brettanomyces* yeasts have been recognized as responsible of acetic acid production and low alcohol synthesis in alcohol production plants as well as of off-flavors production in winemaking. Until now, very few data are available on the requirements of this yeast so that its control is very difficult. Pure cultures and coculture (with *Saccharomyces)* were made under different conditions. The ability of *Brettanomyces*, (Custer positive yeast), to produce acetic acid and alcohol was shown as oxygen supply dependent. Above a given level of oxygen the glucose consumption was inhibited. It was shown that the acetic acid produced by *Brettanomyces* was not the only cause of the decline of *Saccharomyces* yeasts in the polluted fermentation tanks. These yeasts were proved to be able to develop at any step of the winemaking process : alcoholic and malo-lactic fermentations, ageing in cask and bottle. It was shown that these yeasts were very resistant to the preservatives generally used. At least, it was observed that these yeasts presented very different morphological types, depending on the culture conditions and also some metabolic specificities able to explain their behaviour in coculture with *Saccharomyces*. As a conclusion, our observations gave us a way to prevent the development of these undesirable yeasts during alcoholic fermentations of beet molasses (alcohol production) and a better understanding of their action in winemaking. In this field, more attempts are needed to explain the synthesis of off-flavours by a steady state population of *Brettanomyces*.

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1. Grossmann, M.K., Schneider, I., Huehn, T., Remize, F., Dequin, S. 2000. Effects of enhanced glycerol production on yeast activity and fermentation flavour; XXVème Congrès Mondial de la Vigne et du Vin, Congressbook, Paris 19-23 June 2000, pp. 61-67.

Glycerol, side product of alcoholic fermentation, influences in a positively regarded way the intensity of body as well as of mouth feel of wines. Apart from the effects exerted by the amount of glycerol that is secreted into the fermenting must, we could show that the intracellular (internal) glycerol is obviously of striking importance for the overall physiological yeast activity. The internal glycerol content in wine yeast was inversely correlated with fermentation temperature. That means that yeast strains fermenting well at low temperatures had significantly higher amounts of internal glycerol. Others that were obviously not able to synthesize or retain glycerol under cold conditions ran into lagging or stuck fermentations. Flexible response of accumulating internal yeast glycerol turned out to act as a potent stress minimising compound counterbalancing harmful effects of low temperatures, high ethanol content and low nutrient supply. Remize and Dequin found that the activity of glycerol-3-phosphate dehydrogenase, coded by the yeast gene *GPD1*, is of major importance for the production of glycerol. They also showed that overexpression of *GPD1* could deliver up to 20 g/L glycerol in the resulting wines. It was also shown that introduction of *GPD1* harbouring plasmids increased fermentation capacity. This was very pronounced in yeast strains which are known as so called „slow fermenting yeasts“.The overproduction of glycerol caused a bottle neck in the final reducing step of alcoholic fermentation with a high transient accumulation of acetaldehyde and increased production of acetic acid. We investigated therefore how this unwanted accumulation can be counteracted and if or how volatile compounds forming the fermentation bouquet are affected by this metabolic change. Gaschromatographic analysis (GC-MSD) elaborated the amounts of acetic acid esters, fatty acid esters and fatty acids. Although acetaldehyde or closely related compounds were affected esters of aromatic importance stayed within normal concentration range. We could also show that addition of reductive yeast biomass at the end of fermentation strongly decreased the acetaldehyde concentration. Another promising approach to reduce the level of acetate produced was to delete the gene *ALD6* coding for cytoplasmic acetaldehyde dehydrogenase.

2. Rauhut, D., Riegelhofer, M., Ottes, G., Weisbrod, A., Hagemann, O., Glowacz, E. , Löhnertz, O., Grossmann, M. 2000. Investigation of nutrient supply and vitality of yeasts leading to quality improvement of wines and sparkling wines; XXVème Congrès Mondial de la Vigne et du Vin, Congressbook, Paris 19-23 June 2000, pp. 101-106.

A monitoring of nutrient requirements of commercial wine and sparkling wine yeasts seems to be necessary, because an increase of sluggish and stuck fermentations combined with the occurrence of off-flavours was noticeable during the last vintages. Furthermore, the effect of various fermentation activators was proved on fermentation activity and quality of wine and sparkling wine. Using results of broad researches, it was possible to develop artificial musts and wines using a synthetic base medium and nitrogen analysis (amino acids, ammonium compounds) of must and wines from stressed and non stressed vineyards of different

sites and vintages. In this way, it was possible to find out in a reproducible way the nutrient requirements of different yeast strains within the species *Saccharomyces cerevisiae*. In future, it will be possible to examine different impacts of must and base wine treatment, yeast inoculation quantity, temperature, fermentation course etc. under standard conditions using these media. It was possible to show that the permissible maximum quantities for thiamine and ammonium salt additives are adequate for the majority of the tested yeast strains and in case of non stressed musts and base wines. Slow fermenting yeasts present an exception thereto. The addition of yeast cell hull preparations presents a possibility to compensate deficits in nutrient poor musts and base wines and consequently fermentation safety increases. It must be pointed out that as consequence of the use of these preparations sensory negative impacts can result. New production procedures could possibly remedy this disadvantage.Also the addition of inactive yeasts and mannoproteines advanced the fermentation activity, but different effects on the flavour of wines and sparkling wines were noticeable. 20 components of a synthetic base medium were examined on their significance with regard to improvement of fermentation activity. It was shown that the quantity of mineral salts required by yeasts, even in slightly stressed base wines, were available in a sufficient concentration. In extreme stress situations a need of phosphate can occur. Because of this, it is recommended to use diammonium hydrogen phosphate instead of ammonium sulphate already when adding ammonium salt additives to the must. Thiamine represents one of the most important factors with regard to stabilising fermentation activity for the cell-physiological area which has nothing to do with amino acids' requirements and protein biosynthesis. Especially yeasts known as being vital and fermentation active can manage broadly after addition of thiamine additives and ammonium salt even at difficult fermentation conditions. Slow fermenting yeasts have large nutrient requirements, especially a large need of micro nutrient substances (Pyridoxine, p-aminobenzoic acid), consequently their sensory desirable aroma properties can only become visible in musts and base wines rich in nutrients.

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Yeast isolates from raw and processed poultry products were characterized usingPCR amplification of the internally transcribed spacer (ITS) 5.8S ribosomal DNA region (ITS-PCR), restriction analysis of amplified products, randomly amplified polymorphic DNA (RAPD) analysis, and pilsed-field gel electrophoresis (PFGE). ITS-PCR resulted in single fragments of 350 and 650 bp, respectively, from eight strains of *Yarrowia lipolytica* and seven strains of *Candida zeylanoides.* Digestion of amplicons with *Hinf*I and *Hae*III produced two fragments of 200 and 150 bp from *Y. lipolytica* and three fragments of 350, 150, and 100 bp from *C. zeylanoides,* respectively. Although these fragments showed species-specific patterns and confirmed species identification, characterization did not enable intraspecies typing. Contour-clamped heterogenous electric field PFGE separated chromosomal DNA of *Y. lipolytica* into three to five bands, most larger than 2 Mbp, whereas six to eight bands in the range of 750 to 2,200 bp were obtained from *C. zeylanoides.* Karyotypes of both yeasts showed different polymorphic patterns among strains. RAPD analysis, using enrerobacterial repetitive intergeneric sequences as primers, discriminated between strains within the same species. Cluster analysis of patterns formed groups that correlated with the source of isolation. For ITS-PCR, extraction of DNA by boiling yeast cells was successfully used.

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A study was undertaken to determine populations and profiles of yeast species on fresh and processed poultry products upon purchase and after storage at 5ºC until shelf life expiration, and to assess the potential role of these yeasts in product spoilage. Initial populations of yeasts (log_{10} cfu/g) ranged from less than 1 to 2.89, and increased during storage to 0.37-5.06, indicating the presence of psychrotrophic species. Isolates (152 strains) of yeasts consisted of 12 species. *Yarrowia lipolytica* and *Candida zeylanoides* were predominant, making up 39 and 26% of the isolates, respectively. Six different species of basidiomycetous yeasts representing 24% of the isolates were identified. Most *Y. lipolytica* strains showed strong proteolytic and lipolytic activities, whwreas *C. zeylanoides* was weakly lipolytic. Results suggest that these yeasts may play a more prominent role than previously recognized in the spoilage of fresh and processed poultry stored at 5ºC.

3. Ismail, S.A.S., Deak, T., El-Rahman, H.A., Yassien, M.A.M., Beuchat, L.R. 2001. Effectiveness of immersion treatments with acids, trisodium phosphate, and herb decoctions in reducing populations of *Yarrowia lipolytica* and naturally occurring aerobic microorganisms on raw chicken. Int. J. Food Microbiol. **64**: 13-19.

Raw chicken wings inoculated with a mixture of five strains of *Yarrowia lipolytica* isolated from raw poultry were dipped in solutions containing 2, 5, or 8% lactic acid, 2% lactic acid containing 0.2, 0.4, or 0.8% potassium sorbate or sodium benzoate, and 4, 8, or 12% trisodium phosphate solutions. Immerison of wings in 2% lactic acid (with or without 0.2% preservatives) or 4% trisodium phosphate caused a significant reduction in numbers of *Y. lipolytica* and aerobic microorganisms. Treatments with 2% lactic acid or 8% trisodium phosphate reduced numbers of *Y. lipolytica* by 1.47 and 0.65 log_{10} cfu/g, respectively, and aerobic microorganisms by 2.60 and 1.21 log_{10} cfu/g, respectively. Growth of *Y. lipolytica* on wings stored at 5oC up to 9 days, however, was not affected by these treatments. Significant reductions in the populations occurred in 100% basil, marjoram, sage, and thyme decoctions, but not in oregano or rosemary. The small, temporary decreases in populations did not control growth during storage.

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1. Porro, D., Brambilla, L., Ranzi, B.M., Martegani, E., Alberghina, L. 1995. Development of metabolically engineered *Saccharomyces cerevisiae* cells for the production of lactic acid. Biotechnol. Prog. **11**:294-298.

Interesting challenges from metabolically engineered *Saccharomyces cerevisiae* cells arise from the opportunity to obtain yeast strains useful for the production of chemical(s). In this paper, we describe the accumulation of lactic acid in the culture medium of growing, engineered yeast cells expressing a mammalian lactate dehydrogenase gene (LDH-A). High and reproducible productions (20 g/L) and productivities (up to 11 g/L/h) of lactic acid have been obtained by modulating the physiological growth conditions. Since yeast cells are acid tolerant and survive at very low pH values, the production of lactate can be avoided.

2. Compagno, C., Porro, D., Smeraldi, C., Ranzi, B.M. 1995. Fermentation of whey and starch from transformed *Saccharomyces cerevisiae* cells. Appl. Microbiol. Biotechnol. **43**: 822-825.

Among the main agro-industrial wastes, whey and starch are of prime importance. In previous work we showed that strains of *Saccharomyces cerevisiae* transformed with the episomal pM1 allow production of yeast biomass and ethanol from whey/lactose. Ethanol production from whey and derivatives has been improved in computer-controlled bioreactors, while fermentation studies showed that the composition of the medium greatly modulates the productivity (g ethanol produced/l in 1 h of fermentation). A yeast strain for the simultaneous utilization of lactose and starch has also been developed. Biotechnological perspectives are discussed.

3. Porro, D., Ranzi, B.M., Alberghina, L. 1998. Development of a New Flow Cytometric Procedure for the Determination of Single-Cell Growth Properties in Asynchronous *Saccharomyces cerevisiae* Populations. Food Technol. Biotechnol. **36**: 325-332.

A novel flow cytometric procedure has been developed with the aim of obtaining growth properties of individual *Saccharomyces cerevisiae* cells in asynchronous cultures. The method is based on labelling of the celi wall with FITC-conjugated to Concanavalin A and detection of the single cell fluorescence with flow cytometry after exposing the cells to growth conditions. Because the formation of new cell wall material in budded cells is restricted to the bud tip, exposure of the stained cells to growth conditions results in three cell types: (i) stained cells, (ii) partially stained cells, and (iii) unstained cells. This flrst staining has been coupled to the determination of the protein content of the individual cells, which gives a good estimation of the cell size. Analysis of the double staining patterns over time permits determination of cell age and specific growth rate of individual cell cohorts. The procedure has been tested with yeast cell populations growing at dfferent growth rates.

4. Brambilla, L., Balzani, D., Compagno, C., Carrera, V., van Dijken, J.P., Pronk, J. T., Ranzi, B.M., Alberghina, L., Porro, D. 1999. NADH reoxidation does not control glycolytic flux during exposure of respiring *S. cerevisiae* cultures to glucose excess. FEMS Microbiol. Lett. **171**: 133-140.

Introduction of the *Lactobacillus casei* lactate dehydrogenase (LDH) gene into *Saccharomyces cerevisiae* under the control of the TPI1 promoter yielded high LDH levels in batch and chemostat cultures. LDH expression did not affect the dilution rate above which respiro-fermentative metabolism occurred (Dc) in aerobic, glucose-limited chemostats. Above Dc, the LDH-expressing strain produced both ethanol and lactate, but its overall fermentation rate was the same as in wild-type cultures. Exposure of respiring, LDH-expressing cultures to glucose excess triggered simultaneous ethanol and lactate production. However, the specific glucose consumption rate was not affected, indicating that NADH reoxidation does not control glycolytic flux under these conditions.

5. Porro, D., Bianchi, M. M., Brambilla, L., Meneghini, R., Balzani, D., Carrera, V., Lievense, J., Liu, C., Ranzi, B. M., Frontali, L., Alberghina, L. 1999. Replacement of a metabolic pathway for large scale production of lactic acid from engineered yeasts. Appl. Environ. Microbiol. **65** : 4211-4215.

Interest in the production of L-(+)-lactic acid is presently growing in relation to its applications in the synthesis of biodegradable polymer materials. With the aim of obtaining efficient production and high productivity, we introduced the bovine L-lactate dehydrogenase gene (LDH) into a wild-type *Kluyveromyces lactis* yeast strain. The observed lactic acid production was not satisfactory due to the continued coproduction of ethanol. A further restructuring of the cellular metabolism was obtained by introducing the LDH gene into a *K. lactis* strain in which the unique pyruvate decarboxylase gene had been deleted. With this modified strain, in which lactic fermentation substituted completely for the pathway leading to the production of ethanol, we obtained concentrations, productivities, and yields of lactic acid as high as 109 g $liter^{-1}$, 0.91 g $liter^{-1}$ h^{-1}, and 1.19 mol per mole of glucose consumed, respectively. The organic acid was also produced at pH levels lower than those usual for bacterial processes.

6. Compagno, C., Brambilla, L., Capitanio, D., Boschi F., Ranzi B. M., Porro, D. 2001. Alterations of the glucose metabolism in a triose phosphate isomerase-negative *Saccharomyces cerevisiae* mutant. Yeast **18**: 663–670.

The absence of triose phosphate isomerase activity causes an accumulation of only one of the two trioses, dihydroxyacetone phosphate, and this produces a shift in the final product of glucose catabolism from ethanol to glycerol. Alterations of glucose metabolism imposed by the deletion of the TPI1 gene in *Saccharomyces cerevisiae* were studied in batch and continuous cultures. The tpi1 null mutant was unable to grow on glucose as the sole carbon source. The addition of ethanol or acetate in media containing glucose, but also raffinose or galactose, relieved this effect in batch cultivation, suggesting that the Crabtree effect is not the primary cause for the mutant's impaired growth on glucose. The addition of an energy source like formic acid restored glucose utilization, suggesting that a NADH/energy shortage in the delta tpi1 mutant could be a cause of the impaired growth on glucose. The amount of glycerol production in the tpi1 mutant could represent a good indicator of the fraction of carbon source channelled through glycolysis. Data obtained in continuous cultures on mixed substrates indicated that different contributions of glycolysis and gluconeogenesis, as well as of the HMP pathway, to glucose utilization by the tpi1 mutant may occur in relation to the fraction of ethanol present in the media.

7. Merico, A., Rodrigues, F., Côrte-Real, M., Porro, D., Ranzi, B.M., Compagno, C. 2001. Isolation and sequence analysis of the gene encoding triose phosphate isomerase from *Zygosaccharomyces bailii.* In press in Yeast.

The ZbTPI1 gene encoding triose phosphate isomerase (TIM) was cloned from a *Zygosaccharomyces bailii* genomic library by complementation of the *Saccharomyces cerevisiae* tpi1 mutant strain. The nucleotide sequence of a 1.5 kb fragment showed an open reading frame (ORF) of 746 bp, encoding a protein of 248 amino acid residues. The deduced amino acid sequence shares a high degree of homology with TIMs from other yeast species, including some highly conserved regions. The analysis of the promoter sequence of the ZbTPI1 revealed the presence of putative motifs known to have regulatory functions in *S. cerevisiae*. The GenBank Accession No. of ZbTPI1 is AF325852.

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1. Compagno, C., Boschi, F., Ranzi, B.M. 1996. Glycerol production in a triose phosphate isomerase deficient mutant of *Saccharomyces cerevisiae*" Biotechnol. Progress **19**:30-34.

In recent years many attempts have been made to improve biotechnological glycerol production for commercial applications. Glycerol is an important component for taste of beverage and the improvement of the brewing strains was performed in order to increase glycerol production. The absence of triose phosphate isomerase activity is known to cause an accumulation of only one of the two triose, the dyhidroxyacetone phosphate (DHAP). In this paper we show that in a *tpi1* mutant the lack of triose phosphate isomerase produces a deep alteration in the glucose metabolism. The most remarkable effect is that the final product of glucose catabolism is glycerol instead of ethanol. Some strategies for the production of glycerol are described.

2. Compagno, C., Boschi, F., Dal Effe, A., Porro, D., Ranzi, B. M. 1999. Isolation, nucleotide sequence and physiological relevance of the gene encoding triose phosphate isomerase from *Kluyveromyces lactis*. Appl. Environ. Microbiol. **65**: 4216-4219.

Glycerol is an important component for taste of beverage and the improvement of the brewing strains was performed in order to increase glycerol production. Lack of triose phosphate isomerase activity (TIM) is of special interest because this enzyme works at the first and main branched point of the glycolitic flux. The absence of triose phosphate isomerase activity is known to cause an accumulation of only one of the two triose, the dyhidroxyacetone phosphate (DHAP). In a *Saccharomyces cerevisiae* Δ*tpi1* mutant the lack of triose phosphate isomerase produces a deep alteration in the glucose metabolism. The most remarkable effect is that the final product of glucose catabolism is glycerol instead of ethanol. In this paper we report the cloning and sequencing of the gene encoding for triose phosphate isomerase from the dairy yeast *Kluyveromyces lactis*. We show that the *K. lactis* enzyme is the shortest TIM known as yet. Unlike *Saccaromyces cerevisiae,* the *K. lactis* mutant strain was found to be able to grow on glucose. Preliminary experiments of bioconversion indicated that, like the *S. cerevisiae* TIM deficient strain, the *K. lactis* TIM deficient strain is able to produce glycerol with high yield.

3. Merico Rodrigues, F., Côrte-Real, M., Porro, D., Ranzi, B. M., Compagno, C. 2001. Isolation and sequence analysis of the gene encoding triose phosphate isomerase from *Zygosaccharomyces bailii*. Yeast: in press.

On account of the biodiversity existing among yeasts, in the latest years attention has been focused on other yeast genera that displayed peculiar traits more suitable for industrial applications. In this context, *Zygosaccharomyces* species, closely related to *Saccharomyces*, are of particular interest due to their ability to survive under various stress and environmental conditions. *Z. bailii* is known as a food spoilage yeast for its ability to grow on mixtures of sugars in the presence of high concentration of acetic acid, and low pH. However, sugar metabolism, in this species has not been so far extensively studied. In this sense, it was attempted to isolate in *Z. bailii* the gene encoding TIM that will allow to study sugar metabolism and glycerol production. The *ZbTPI1* gene encoding triose phosphate isomerase (TIM) was cloned from a *Zygosaccharomyces bailii* genomic library by complementation of the *Saccharomyces cerevisiae tpi1* mutant strain. The nucleotide sequence of a 1.5 kb fragment showed an open reading frame (ORF) of 746 bp, encoding a protein of 248 amino acid residues. The deduced amino acid sequence share a high degree of homology with the TIMs of other yeast species and highly conserved regions. The analysis of the promoter sequence of the *ZbTPI1* revelead the presence of motifs known to have regulatory function in *S. cerevisiae*.

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1. De Philippis, R., Bastianini, A., Granchi, L., Messini, A., Vincenzini, M. 1999. Phospholipids and fatty acyl composition of S*accharomyces cerevisiae* as affected by oxygen and low growth temperature. Annali Microbiolol.Enzimol. **49**:145-154.

A strain of *Saccharomyces cerevisiae*, previously shown to be able to complete the fermentation of grape must at low temperature, was grown in the presence and in the absence of oxygen at 10 and 25°C in order to investigate about the effects of low growth temperature on the phospholipids (PLs) and on the fatty acyl (FA) composition of the yeast cells. Both anaerobic and aerobic cells grown at the lower temperature showed a much higher amount of total PLs due to the increased amount of phosphatidylethanolamine, phosphatidylinositol and phosphatidylcholine, whereas the cellular concentration of the other PLs, namely phosphatidylglycerol and phosphatidylserine, was not significantly modified. The amount of unsaturated FAs of whole cells was almost unaffected by the lower growth temperature, but a very significant increase of their quantity was observed in the phospholipidic fraction of cells grown at 10°C. No significant modifications in the mean fatty acid chain length of both whole cells and PLs were observed, demonstrating that this parameter is practically temperature-independent. These results point out that low growth temperatures stimulate both the synthesis of PLs and the increase of their unsaturation degree, without significantly affecting the amount of unsaturated FAs of whole cells.

2. Granchi, L., Bosco, M., Messini, A., Vincenzini, M. 1999. Rapid detection and quantification of yeast species during spontaneous wine fermentation by PCR-RFLP analysis of the rDNA ITS region. J. Appl. Microbiol. **87**: 949-956.

PCR-RFLP analysis of the rDNA-ITS region with five endonucleases was applied to 174 yeast strains belonging to 30 species of oenological significance and including 27 type strains in order to develop a rapid protocol for yeast identification. PCR-RFLP patterns resulted species-specific with the exception of teleomorphic and anamorphic forms. According to these findings, a protocol taking about 30 hours was set up and applied for the detection and quantification of yeast species occurring in the course of a spontaneous wine fermentation at industrial level. This protocol consisted of the following steps: 1) plating of must or wine sample; 2) 24 hours of plate incubation; 3) sampling of all microcolonies (90 or more/plate); 4) cell lysis by heating at 80°C for 10 min; 5) amplification of rITS and direct restriction analysis 6) separation of the restriction fragments on 3.5 % agarose gel electrophoresis using chilled buffer; 7) digital acquisition of restriction patterns images and comparison with our database of yeast rITS PCR-RFLP patterns. A representative sample of colonies from WL agar plates were identified by traditional methods, too. Both procedures gave identical responses. However, PCR-RFLP analysis furnished more precise quantitative data, proving to be a reliable and simple method for monitoring the development of the yeast community throughout wine fermentation and its storage. The timely knowledge on which yeasts are transforming grape must into wine should give a rationale for any technological operation feasible in the cellar.

3. Granchi, L., Borghini, F., Viti, C., Vincenzini, M. 2000. On the origin and biodiversity of *Saccharomyces cerevisiae* strains dominating the spontaneous wine fermentation. Symposium Book ISY 2000. "The rising power of yeasts in science and industry", 27 August-1 September 2000, Papendal, Arnhem(NL), pp. 284-285.

The origin of the strain(s) of *Saccharomyces cerevisiae* dominating the spontaneous wine fermentation is controversial, the primary source possibly being the winery and its equipments or the vineyard. In this work the search for *S. cerevisiae* was carried out in the vineyard (all grapes from a single vine were collected, crushed and the resultant must was allowed to ferment in the

laboratory), in the winery before the vintage (the inside of a wooden tank was scrubbed by swabs) and in the winery during the spontaneous wine fermentation occurring in the same tank. No *Saccharomyces* strain was isolated from the grapes of the single vine nor during the grape juice fermentation performed in the laboratory. On the contrary, *S. cerevisiae* isolates were recovered both from the tank before the vintage and at different stages of the wine fermentation. The isolates were differentiated by mitochondrial DNA (mtDNA) restriction analysis and *S. cerevisiae* strains showing different mtDNA restriction patterns were characterized for some of their oenological properties. The *S. cerevisiae* strains dominating the middle and the end of fermentation showed profiles different from those of the strains isolated from the tank before the vintage. Indeed, the dominant profile in the tank was only rarely found during the wine fermentation in the same tank. The highest number of patterns was obtained at the end of fermentation, but, in this case, the dominant strain was the same as that dominating the middle phase of fermentation. As concerns the phenotypic characterization, no marked differences were found among the strains so that the observed molecular diversity did not find confirmation in a phenotypic diversity. These results don't provide univocal information about the origin of the *S. cerevisiae* strains dominating the wine fermentation. However, on the basis of the mtDNA analysis, they resulted different from those isolated from the tank. If they originated from the vineyard, they would have been present at very low cell populations and probably not uniformly distributed, as it was demonstrated by the incomplete fermentation of grapes collected from a single vine plant.

4. Viti, C., Forni, D., Ventura, S., Messini, A., Materassi, R., Giovannetti, L. 2000 Characterisation and typing of *Saccharomyces* strains by DNA fingerprinting. Annal. Microbiol. **50**:191-203.

Three different molecular methods, total DNA restriction profile analysis, restriction profile of mitochondrial DNA (mtDNA) and Southern hybridisation of mtDNA, were used to characterise Italian wine yeast strains previously identified using conventional taxonomic techniques. Total DNA restriction profile analysis allowed the typing of all strains and showed that they constitute two well separated genomic taxa, one including the type strain of *S. cerevisiae* and the other the type strain of *S. bayanus*. The data obtained by analysing mtDNA restriction profiles and mtDNA Southern hybridisation were consistent with results of total DNA restriction profile analysis. This permitted the taxonomic assignment of Italian wine yeast strains and the determination of the interspecific genomic relatedness.

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1. Romano, P., Palla, G., Caligiani, A., Brandolini, V. 2000. Evaluation of stereoisomers of 2,3-butanediol and acetoin to differentiate *Saccharomyces cerevisiae* and *Kloeckera apiculata* wine strains. Biotechnol. Lett. **22**: 1947-1951.

(R)/(S) ratios of acetoin were always higher in wines obtained by *S.cerevisiae* than in those by *K.apiculata*. A significantly different behaviour was determined between the two species as regards contents and ratios of 2,3-butanediols: *S.cerevisiae* produced more (R,R)-2,3-butanediol (about 80%), whereas *K.apiculata* more meso-form (about 90%).

2. Romano, P., Caruso, M., Fiore,C., Paraggio, M., Capace, A.,.Lipani, G. 2000. Characterisation of indigenous yeasts associated with Aglianico of Vulture grape variety cultured in Basilicata. Proc. 10th ISY, Papendal, Arnhem (The Netherlands), 27 Aug.-1 Sept.

A total of 200 yeasts isolated from different grape and must samples of Aglianico of Vulture, the traditional wine of Basilicata region, were used: 100 strains of S*accharomyces cerevisiae* from the end of the fermentation process and 100 strains of *Kloeckera apiculata* from the first days of fermentation. The strains of the two species were studies for phenotypic and genotypic characteristics, by the combined use of physiological, biochemical and molecular analyses. Regarding *S.cerevisiae*, tested for, the majority of the strains were classified as resistant for all the parameters considered (resistance to sulphur dioxide and copper and ethanol tolerance). Regarding *K.apiculata* strains, significant strain variability in proteolytic activity was found. The strains of the two species were tested in microfermentations and at the end of the process the content in some by-products was determined by gas chromatography. Even though considerable strain variability was found in both species, the metabolic behaviour discriminated between *S.cerevisiae* and *K.apiculata*. In order to determine the genetic polymorphism, some strains, as representatives of the two species, were further characterized by PCR fingerprinting with microsatellite oligonucleotide primers $(GTG)_5$ and $(GAG)_5$, *S.cerevisiae* Aglianico strains exhibited a significant genetic variability, whereas *K.apiculata* strains exhibited a more uniform molecular pattern.

3. Romano, P., Paraggio, M., Caruso, M., Lipani, G., Capace, A., Fiore, C., Salzano, G., Ricciardi, A., Raso, F. 2000. *Saccharomyces cerevisiae* indigenous population and its effect on the organoleptic characteristics of Aglianico wine. Proc. Int. Vitic. Enol. Congress, Capetown (Sud Africa), 8-10 Nov.

One hundred and fifteen *Saccharomyces cerevisiae* strains, isolated from Aglianico of Vulture in Basilicata (Italy), were characterized for traits of technological interest in winemaking and for genetic polymorphism. Both analyses revealed a considerable degree of variability, indicating a heterogeneous natural population. Among technological strain characteristics, we determined fermentation efficiency, resistance to sulphur dioxide and to copper, ethanol tolerance and production of fermentation secondary compounds involved in the aroma and taste of alcoholic beverages. All the strains exhibited a general high fermentation activity, reaching the maximal weight loss after ten days. The majority of the strains were able to tolerate a considerable ethanol concentration and exhibited also a high SO_2 resistance, at least to 175 ppm. Wines obtained by microvinification with the Aglianico strains did not differ in the production levels of n-propanol, active amyl alcohol and ethyl acetate, whereas isobutanol, isoamyl alcohol and acetaldehyde were formed with a wide variability. A good discrimination among Aglianico strains was observed with the microsatellite primer $(GTG)_5$, but strain genetic pattern was not related to strain phenotypic characteristics. Three strains, exhibiting different responses to the parameters considered, were tested for tetrad analysis. The progeny corresponded to the parental strains and was homozygous for fermentation efficiency, resistance to sulphur dioxide and to copper and ethanol tolerance. On the contrary a metabolic variability was observed in the progeny with segregation at one to three compounds.

4. Romano, P., Paraggio, M. Monteleone, E., Fiore, C., Lipani, G., Caruso, M., Capece ,A. Ricciardi, A. 2001. Starter cultures for wine quality and tipicality : desirability index as A selective tool. Ind. Bevande **30**: 11-17.

Two hundreds strains of *Saccharomyces cerevisiae*, isolated from Basilicata grape musts, were characterized by applying a methodological approach, based on wine typicality characteristics as a selective tool for starter cultures. Aglianico of Vulture, the main and typical wine of the Basilicata region, was used in this study. The comparision between metabolic profile of *S. cerevisiae* strains and that of commercial Aglianico wines allowed the selection of some strains potentially more suitable, at least for the traits considered, to perform the fermentation and to maintain the individual characteristics of Aglianico wine. The selected strains were tested for genetic analysis and only one strain exhibited a good genetic stability for the selection variables chosen. Pilot scale fermentation in cellar with the selected strain gave reproducible results, which were consistent with those obtained on laboratory scale. Sensorial evaluation of experimental wine resulted in an evident correspondence with the average profile of some commercial Aglianico wines.

5. Sipiczki, M., Romano, P., Lipani, G., Miklos, I., Antunovics, Z. 2001. Analysis of yeasts derived from natural fermentation in a Tokaj winery. Antonie van Leeuwen. **79**: 97-105.

The diversity of yeast flora was investigated in a spontaneously fermenting sweet white wine in a Tokaj winery. The non-*Saccharomyces* yeasts dominating the first phase of fermentation were soon replaced by a heterogeneous *Saccharomyces* population, which then became dominated by *Saccharomyces bayanus*. Three *Saccharomyces* sensu stricto strains isolated from various phases of fermentation were tested for genetic stability, optimum growth temperature, tolerance to sulphur dioxide, copper and ethanol as well as for the ability to produce hydrogen sulphide and various secondary metabolites known to affect the organoleptic properties of wines. The analysis of the single-spore cultures derived from spores of dissected asci revealed high stability of electrophoretic karyotypes and various degrees of heterozygosity for mating-types, the fermentation of galactose and the production of metabolic by-products. The production levels of the by-products did not segregate in a 2:2 fashion, suggesting that the synthesis of these compounds is under polygenic control.

6. Paraggio, M., Fiore, C., Romano, P. 2001. Yeast flocculation influence on volatile bouquet substances of wine. Alcologia:000-000.

Forty flocculent strains of *Saccharomyces cerevisiae* were tested in Trebbiano grape must at 18°C for the floc fomation and the production of higher alcohols, acetic acid, ethyl acetate and acetaldehyde. A significant variability in strain flocculation degree was determined, allowing the discrimination of the strains in 6 different groups. Of the 40 strains, 12 possessed the maximum flocculation level (F6), yielding on the flask bottom a coherent layer, which, after shaking, did not yielded torbidity. As regards the fermentation behaviour, all the strains exhibited a similar fermentation energy and no significant differences were recorded between the different flocculent phenotypes. Regarding by-product formation, the flocculent strains exhibited a uniform behaviour in the production levels of acetaldehyde, ethyl acetate, whereas n-propanol and isobutanol were produced with significative variability, respectively from 5.98 to 26.18 mg/L for n-propanol and from 9.45 to 34.38 mg/l for isobutanol. Other compounds, such as isoamyl alcohol and acetic acid, were formed with a wide variability, the amounts produced depending on the strain which performed grape must fermentation. Isoamyl alcohol was formed from 47.15 to 127.86 mg/L and acetic acid from 103 to 694.56 mg/l. The results of this work revealed that strain metabolic variability led to the individuation of different behaviours correlated with differing levels of flocculation. The major difference was observed in the production of isoamyl alcohol and acetic acid, the low productions being always correlated to a high degree of strain flocculation. It appears that the choice of the flocculent strain determines not only a major efficiency in the technological removal of yeast cells, but also different proportions and concentrations of some volatile compounds. Studies are in progress in our laboratory to ascertain on pilot scale fermentation the correlation of flocculent phenotypes with strain aromatic properties.

7. Comi, G., Romano, P., Cocolin, L., Fiore, C. 2001. Characterization of *Kloeckera apiculata* strains from Friuli region in North Italy. World J. Microbiol. Biotechnol. **17**:000-000.

Forty nine strains of *Kloeckera apiculata*, isolated from Friuli region in Italy, were differentiated on the basis of fermentation behaviour and production of secondary compounds in two different grape musts at 18°C. The isolates exhibited a controlled production of acetic acid, only in few cases more that 1 g/l. In Moscato grape must the strains exhibited a more uniform behaviour for the production of higher alcohols, ethyl acetate and acetoin than in red grapes. In general, higher levels of ethanol, glycerol and acetic acid were produced in red grape must fermentation. Apiculate strains behaved differently in the two musts, with different metabolic phenotypes dominating the fermentation process. The existence of different metabolic phenotypes correlated to the must composition underlines the need to perform a selection of indigenous apiculate yeasts to obtain the desired consistent products.

8. Romano ,P., Ricciardi, A., Salzano, G. , Suzzi, G. 2001. Yeasts from Water Buffalo Mozzarella, a traditional cheese of the Mediterranean area. In press on Int. J. Food Microbiol.: in press.

Countries of the Mediterranean area are characterized by production of artisanal cheeses, obtained from goat, sheep, cow and buffalo raw milk. The numbers and species of yeasts in the different cheeses are variable, but some species are more frequently detected than others. *Kluyveromyces marxianus*, *K. lactis* with their anamorph, *Candida kefir*, *Debaryomyces hansenii* and *C. famata*, *C. colliculosa* and *C. catenulata* are dominant species in several cheeses. However, the non-lactose fermenting *Saccharomyces cerevisiae* is often detected in pasta filata cheeses, such us Water Buffalo Mozzarella or Cacio Cavallo Podolico. Recently, a comprehensive study of yeasts isolated from Mozzarella cheese produced in Basilicata (Southern Italy) has been carried out. The study has focused on lactose and/or galactose fermenting species (*Kluyveromyces* and *Saccharomyces*) to evaluate their role on the functional and sensory properties of the product. End products in milk were evaluated and the biodiversity (in terms of production of sulphur dioxide, higher alcools, ethyl acetate, and acetaldehyde) was studied. In particular, *S. cerevisiae* strains from Water Buffalo Mozzarella cheese, compared to strains isolated from different habitats, such as wine, exhibited considerable difference in the production of some volatile compounds. The intraspecific diversity observed could be related to the particular microhabitat of *S. cerevisiae* occurring in whey and cheese of water buffalo milk.

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1. Blom, J., De Mattos, M.J., Grivell, L.A. 2000. Redirection of the respiro-fermentative flux distribution in *Saccharomyces cerevisiae* by overexpression of the transcription factor Hap4p. Appl Environ Microbiol **66**(5):1970-1973.

Reduction of aerobic fermentation on sugars by altering the fermentative/oxidative balance is of significant interest for optimization of industrial production of *Saccharomyces cerevisiae*. Glucose control of oxidative metabolism in baker's yeast is partly mediated through transcriptional regulation of the Hap4p subunit of the Hap2/3/4/5p transcriptional activator complex. To alleviate glucose repression of oxidative metabolism, we constructed a yeast strain with constitutively elevated levels of Hap4p. Genetic analysis of expression levels of glucose-repressed genes and analysis of respiratory capacity showed that Hap4p overexpression (partly) relieves glucose repression of respiration. Analysis of the physiological properties of the Hap4p overproducer in batch cultures in fermentors (aerobic, glucose excess) has shown that the metabolism of this strain is more oxidative than in the wild-type strain, resulting in a significant reduced ethanol production and improvement of growth rate and a 40% gain in biomass yield. Our results show that modification of one or more transcriptional regulators can be a powerful and a widely applicable tool for redirection of metabolic fluxes in microorganisms.

2. van Maris, A.J.A., Bakker, B.M., Brandt, M., Boorsma, A., Teixeira de Mattos, M.J., Grivell, L.A., Pronk, J.T., Blom, J. 2001. Modulating the distribution of fluxes among respiration and fermentation by overexpression of *HAP4* in *Saccharomyces cerevisiae*. FEMS Yeast Res. **1**(2): in press.

The tendency of *Saccharomyces cerevisiae* to favour alcoholic fermentation over respiration is a complication in aerobic, biomass-directed applications of this yeast. Overproduction of Hap4p, a positive transcriptional regulator of genes involved in respiratory metabolism, has been reported to positively affect the balance between respiration and fermentation in aerobic glucose-grown batch cultures. In this study, the effects of *HAP4* overexpression have been quantified in the prototrophic *S. cerevisiae* strain CEN.PK113-7D under a variety of growth conditions. In aerobic glucose-limited chemostat cultures, overexpression of *HAP4* increased the specific growth rate at which aerobic fermentation set in by about 10 % relative to the isogenic wild type. Upon relief of glucose-limited conditions, the *HAP4*-overexpressing strain produced slightly less ethanol than the wild-type strain. The effect of Hap4p overproduction was most drastic in aerobic, glucose-grown chemostat cultures in which ammonium was limiting. In such cultures, the biomass yield on glucose was twofold higher than that of the wild type.

Poland: **Institute of Agricultural and Food Biotechnology, Rakowiecka Street 36, D2-532 Warsaw. Communicated by Krystyna Stecka <ztsid@ibprs.pl>.**

1. Piasecka-Józwiak, K., Badocha, E., Grzybowski, R., Stecka, K. 1998. Studies on the influence of baker's yeast Saccharomyces cerevisiae culture medium enrichment with selected biodtymulators on the yeaqst fermentation activity and biomass yield. Prace Instytutów i Laboratoriów Badawczych Przemyslu Spozywczego, 53, s.5

The increase of baker's yeast fermentation activity by enrichment of their culture medium with selected biostimulators was the aim of presented work. It was stated that the enrichment of molasses wort with 600-1200 μg thiamine hydrochloride per litre caused the increase of yeast fermentation activity by 4-12%. Addition of that vitamin at the end of generation II cultivation gave the best effect. In case of the pyridoxine or panthotenic acid addition, applied during cultivation, none improvement of the yeast fermentation activity was obtained. The corn steep liquor addition in amount of 50 ml/l of culture medium slightly increased yeast fermentation activity. The enrichment of molasses wort zinc in amounts of 40 and 80 mg/l (as zinc ions) improved yeast fermentation activity by 6-9%. Addition of 0,65 mg Cu/l (as cooper ions) and 0,55 mg Mn/l (as manganase ions) under applied experimental conditions, caused the increase (about 5%) of yeast fermentation activity.

2. Poplawska, S., Piasecka-Józwiak, K., Grzybowski, R. 1998. The trials to establish the optimal conditions for the cfontinuous culture of the bakery yeast KKP 512. Przemysl Fermentacyjny i Owocowo-Warzywny, Nr 10, s. 49-52.

There were carried out series of experiments to establish optimal parameter of the continuous cultivation of baker's yeast. Goal of this work was to obtain maximum yield and high fermentation activity of the yeast biomass. The fermentation activity of biomass in all kind of trials were stated higher then in yeast produced by fed –batch method. The best yield of yeast was obtained in experiments using molasess medium of sugar concentration 71 g/L, in dilution rate D = 0,12 1/h.

3. Stecka, K.M., Komorowska, A., Mrówka, E., Grzybowski, R.A. 1999. Activation of the baker's yeast autolysis. Pol. J. Food Nutr. Sci. **8** (49) No 2: 227-234.

The activation effect of baker's yeast *Saccharomyces cerevisiae* autolysis was examined under standard conditions with addition of certain compounds: organic acids, vitamins, sugars, inorganic salts, ethanol, and their mixtures. The efficiency of autolysis in the presens of mixed activators (glucose/ethanol/sodium chloride in the concentration of 0.5/1.0/1% by wt.), increased by 50%, or by 80% when 0.15% chitosan solution was applied. These figures relate to the amount of protein expelled in spontaneus autolysis. The highest increase of protein liberated from yeast cells, above 100%, was obtained when the process was activated with organic acids at a concentration of 0.5%.

4. Mrówka, E., Rozmierska, J., Komorowska, A., Stecka, K.M. 1999. Functional properties of selected yeast products. Zywnosc **3**: 7-16.

The functional properties: solubility, fat binding capacity, emulsion capacity and foaming properties of selected yeast products: yeast extracts, yeast protein isolate and hydrolyzate were investigated. Yeast extracts and yeast protein hydrolyzate had a good solubility in the pH range 2 – 9. The solubility of the yeast protein concentrate decreased at the isoelectric point (pH 4). The fat binding capacity FBC of the two yeast extracts was 60% and 80% respectively, and the foam capacity, FC was 300% and 500%. No emulsion capacity was found for the extracts. The yeast protein isolate has a good fat binding capacity FBC=170%, foaming capacity FC =700% and emulsion capacity EC=82%. The functional properties of yeast protein hydrolyzates were: fat binding capacity FBC=72%, foaming capacity FC=600% and emulsion capacity EC= 70%. The good solubility and the functional properties of the selected yeast products show that they can be used in the food industry.

5. Komorowska, A., Mrówka, E., Stecka, K. M., Grzybowski, R.A. 1998. Investigation on the influence of selected inductors on the effectiveness of autolysis of baker's yeast. Pr. Inst. Lab. Bad. Przem. Spoz. **53**: 22-31.

The influence of selected autolysis inductors on the efficiency of releasing of cytoplazmatic components from baker's yeast cells of selected strain after 24-hour autolysis process in 50°C was investigated. The addition of glucose or ethanol to the autolytic suspension increased the amount of released proteins by at least 50%. The mixture of glucose and ethanol gave about 60% increase of the yield of liberated proteins, as compared with spontaneous autolysis. Effect of adding of NaCl and KCl as autolysis inductors on the amount of extracted proteins was highest at the concentration of NaCl - l .0% and KCl - 0,5% and gave the increase of autolysis effectiveness by 20 and 30% respectively, at higher salt concentrations the efficiency decreased. The influence of addition of fresh autolysate of the same strain as inductor of autolysis has been estimated as not significant. It was observed, that the mixture of glucose/ethanol/NaCl at concentrations 0.5/1.0/2.5% by wt. used as autolysis inductor was most efficient. This addition increased the amount of liberated proteins by about 80%.

6. Komorowska, A., Mrówka, E., Stecka, K.M., Grzybowski, R.A. 1998. Influence of chitosan on the amount of released intracellular components from yeast S*accharomyces cerevisiae* in the autolysis process. Pr. Inst. Lab. Bad. Przem. Spoz. **53**: 32-39.

In the autolysis process of baker's yeast performed under standard conditions in 50 °C in time 24 h, using of chitosan as autolysis activator in concentrations from 0.025 to 0.15% by wt. induce the improvement of the efficiency of autolysis. It has been obtained the increase of the amount of released proteins from yeast cells to the autolytic suspension in this conditions, in relation to the amount of protein expelled in spontaneous autolysis. Using of chitosan at the concentration of 0.025% by wt. gave 25% increase of the amount of released proteins and at the concentration 0.015% of chitosan this increase amounted 80%.

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1. Schuller, D., Côrte-Real, M., Leão, C. 2000. A differential medium for the enumeration of the spoilage yeast *Zygosaccharomyces bailii* in wine. J. Food Prot. **63** (11), 1570-1575.

A collection of yeasts, isolated mostly from spoiled wines, was used in order to develop a differential medium for *Zygosaccharomyces bailii.* The 118 selected strains of 21 species differed in their origin and resistance to preservatives and belonged to the genera *Pichia, Torulaspora, Dekkera, Debaryomyces*, *Saccharomycodes, Issatchenkia, Kluyveromyces, Kloeckera*, *Lodderomyces, Schizosaccharomyces, Rhodotorula, Saccharomyces* and *Zygosaccharomyces.* The design of the culture medium was based on the different ability of the various yeast species to grow in a mineral medium with glucose and formic acid (mixed-substrate medium) as the only carbon and energy sources and supplemented with an acid-base indicator. By manipulating the concentration of the acid and the sugar it was possible to select conditions where only *Z. bailii* strains gave rise to alkalization, associated to a color change of the medium (positive response). The final composition of the mixed medium was adjusted as a compromise between the percentage of recovery and selectivity for *Z. bailii.* This was accomplished by the use of pure or mixed cultures of the yeast strains and applying the membrane filtration methodology. The microbiological analysis of two samples of contaminated "Vinho Verde" allowed to conclude that the developed medium can be considered as a differential medium to distinguish *Z. bailii* from other contamination yeasts, with potential application in the microbiological control of wines and probably other beverages and foods.

2. Prudêncio, C., Sansonetty, F., Sousa, M. J., Côrte-Real, M., Leão, C. 2000. Rapid detection of efflux pumps and their relation with drug resistance in yeast cells. Cytometry **39**: 26-35.

Cell drug resistance can be due to the presence of active efflux pumps (AEP). Identification of yeasts with a resistance phenotype is important either from a clinical, agricultural or biotechnological point of view. Rapid and reliable methods to detect AEP can be therefore very useful. Some yeast cells change their staining by calcein-AM, BCECF-AM, rhodamine 123 and $DiOC_5$, when pretreated with verapamil, CCCP or ATP depletion, or when pretreated with specific antimicrobial agents. This fact may be interpreted as an indication of the presence/absence of AEP. Six yeast species were tested with a flow cytometric method (FCM) and an epifluorescence microscopic method (EFM) and other ten species evaluated only by EFM. The minimum inhibitory concentration (MIC) of penconazol, benomyl and cycloheximide for *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*, were determined by growth inhibition on solid medium and were compared to the staining changes detected by FCM. The FCM and the EFM allowed the detection of AEP in all the yeast species tested. High MIC values for a drug were correlated with the presence of at least one AEP indicated by the cytometric data. The FCM revealed to be a robust assay whereas the EFM can be used as a preliminary test. It is possible to identify resistance/sensitivity patterns in yeast cells through cytometric detection methods of different efflux pumping systems.

3. Rodrigues, F, Corte-Real, M, Leao, C, van Dijken, JP, Pronk, JT. 2001. Oxygen Requirements of the Food Spoilage Yeast *Zygosaccharomyces bailii* in Synthetic and Complex Media. Appl. Environ. Microbiol. **67**(5):2123-8.

Most yeast species can ferment sugars to ethanol, but only a few can grow in the complete absence of oxygen. Oxygen availability might, therefore, be a key parameter in spoilage of food caused by fermentative yeasts. In this study, the oxygen requirement and regulation of alcoholic fermentation were studied in batch cultures of the spoilage yeast *Zygosaccharomyces bailii* at a constant pH, pH 3.0. In aerobic, glucose-grown cultures, *Z. bailii* exhibited aerobic alcoholic fermentation similar to that of *Saccharomyces cerevisiae* and other Crabtree-positive yeasts. In anaerobic fermentor cultures grown on a synthetic medium supplemented with glucose, Tween 80, and ergosterol, *S. cerevisiae* exhibited rapid exponential growth. Growth of *Z. bailii* under these conditions was extremely slow and linear. These linear growth kinetics indicate that cell proliferation of *Z. bailii* in the anaerobic fermentors was limited by a constant, low rate of oxygen leakage into the system. Similar results were obtained with the facultatively fermentative yeast Candida utilis. When the same experimental setup was used for anaerobic cultivation, in complex YPD medium, *Z. bailii* exhibited exponential growth and vigorous fermentation, indicating that a nutritional requirement for anaerobic growth was met by complex-medium components. Our results demonstrate that restriction of oxygen entry into foods and beverages, which are rich in nutrients, is not a

promising strategy for preventing growth and gas formation by *Z. bailii*. In contrast to the growth of *Z. bailii*, anaerobic growth of *S. cerevisiae* on complex YPD medium was much slower than growth in synthetic medium, which probably reflected the superior tolerance of the former yeast to organic acids at low pH.

4. Rodrigues, F., van Hemert, M., Steensma, Y. H., Côrte-Real, M., Leão, C. 2001. Red fluorescent protein (DsRed) as a reporter in *Saccharomyces cerevisiae.* J Bacteriol. **183**(12):00-00.

Green fluorescent protein (GFP) is a powerful tool for identifying the sub-cellular localization of proteins and to monitor gene expression. The protein is capable of producing a strong green fluorescence when excited by blue light, without any exogenously added substrate or co-factor. Events inside living cell can thus be visualized in a non-invasive way. For *Saccharomyces cerevisiae*, a series of plasmids has been developed for the expression of N-terminal and C-terminal in-frame fusions with the protein of interest. These so-called pUG vectors have been proven useful for localization and expression studies in this yeast. Recently, fluorescent proteins have been described that emit light with a wavelength different from that of GFP. These comprise blue-, cyan- and yellow-shifted mutants of GFP and the newly isolated DsRed. The latter is a red-emitting fluorescent protein. The longer wavelength of the emitted light minimizes problems associated with light scattering and auto-fluorescence of the cells. Fluorescent proteins with different emission colors are valuable for *in vivo* multi-labeling experiments, allowing co-monitoring several events. In this work, we have focused on the use of the red fluorescent protein DsRed as a reporter in yeast cells. Based on the pUG plasmids, a new set of vectors expressing *DsRed* was constructed, allowing the production of amino-terminal (N-terminal) and carboxyl-terminal (C-terminal) fusion proteins. In addition, we investigated the expression and localization of DsRed either with or without localization signal, in *S. cerevisiae,* by fluorescence and confocal microscopy. Expression of the *DsRed* gene under the control of the *MET25* promoter resulted in circa 90% uniformly stained cells of *S. cerevisiae*. Furthermore, this expression did not result in any toxic effect on cell growth. Addition of an in-frame nuclear localization signal to the *DsRed* gene, targeted fluorescence exclusively to the nucleus, indicating that the protein has no active target sequences in *S. cerevisiae*. Clones expressing red and/or green fluorescent proteins with both cytoplasmic and nuclear localization were obtained. It was shown that double labeling with green and red fluorescent proteins could be used for the study of expression and co-localization of proteins. Our results indicate that *DsRed* can be expressed in *S. cerevisiae* and the protein can be targeted specifically to the nucleus. Finally, we show that cells with nuclei labeled with either red or green fluorescent proteins can be used to follow mating *in vivo*.

5. Rodrigues, F., Zeeman, A. M., Alves, C., Sousa, M.J., Steensma, H.Y., Côrte-Real, M., Leão, C. 2001. Construction of a genomic library of the food spoilage yeast *Zygosaccharomyces bailii* and isolation of the b-isopropylmalate dehydrogenase gene (*ZbLEU2*). Yeast FEMS Res.: in press.

A genomic library of the yeast *Zygosaccharomyces bailii* ISA 1307 was constructed in pRS316, a shuttle vector for *Saccharomyces cerevisiae* and *Escherichia coli*. The library has an average insert size of 6 kb and covers the genome more than twenty times assuming a genome size similar to that of *S. cerevisiae*. This new tool has been successfully used, by us and others, to isolate *Z. bailii* genes. One example is the b-isopropylmalate dehydrogenases gene (*ZbLEU2*) of *Z. bailii*, which was cloned by complementation of a *leu2* mutation in *S. cerevisiae*. An open reading frame, encoding a protein with a molecular weight of 38.7 kDa was found. The nucleotide sequence of *ZbLEU2* and the deduced amino acids sequence showed a significant degree of identity to those of b-isopropylmalate dehydrogenases from several other yeast species. The sequence of *ZbLEU2* has been deposited in the EMBL data library under Accession No. AJ292544.

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1. Domingues, L, Lima, N, Teixeira, JA. 2001. Alcohol production from cheese whey permeate using genetically modified flocculent yeast cells. Biotechnology and Bioengineering **72**: 507-514.

Cheese whey permeate alcoholic fermentation was investigated using a recombinant flocculating *Saccharomyces cerevisiae*, expressing the *LAC4* and *LAC12* genes of *Kluyveromyces marxianus* enabling lactose metabolization. For batch experiments, the use of cheese whey permeate (50 gL^{-1} lactose) as substrate resulted in total lactose consumption and ethanol conversion yield close to the expected theoretical value. When using 2-times concentrated cheese whey permeate, corresponding to 100 gL^{-1} lactose concentration, a fermentation product with 5 % (w/v) alcohol was obtained. For continuous operation studies, a 5.5-L airlift bioreactor was used. The high-cell-density continuous operating bioreactor fed with cheese whey permeate (50 gL^{-1} lactose) allowed for 10 $gL^{-1}h^{-1}$ ethanol productivity (corresponding to 0.45h^{-1} dilution rate), which raises new perspectives for the economic feasibility of whey alcoholic fermentation. For continuous operation fed with 2-times concentrated cheese whey permeate, inhibition of cell flocculation by salts was noticed, which hindered the operation at high cell density.

2. Domingues, L, Vicente, AA, Lima, N, Teixeira, JA. 2000. Applications of yeast flocculation in biotechnological processes. Biotechnology Bioprocess Engineering **5**: 288-305.

This is a review paper dealing with the main aspects associated with yeast flocculation and its application in biotechnological processes. This subject is addressed following three main aspects – the basics of yeast flocculation, the development of "new" flocculating yeast strains and bioreactor development. The paper concludes describing some of the applications of high cell density flocculation bioreactors and discussing potential new uses of these systems.

3. Domingues, L, Lima, N, Teixeira, JA. 2000. Contamination of a high-cell-density continuous bioreactor. Biotechnology and Bioengineering **68**:584-587.

Contamination resistance of an airlift bioreactor operating at continuous high cell density was evaluated. The bioreactor carrying a recombinant flocculent *Saccharomyces cerevisiae* strain operating under steady state at a dilution rate of 0.45 h^{-1}, was contaminated with recombinant *Escherichia coli* cells. The recombinant strain of *Escherichia coli* was engineered to produce GFP (green fluorescent protein) facilitating the contaminant detection. The faster growing *E. coli* strain was washed out of the bioreactor and the recombinant, slower growing flocculating *S. cerevisiae* strain remained as the only species detected in the bioreactor. Flocculation, besides allowing for the realization of high-cell-density systems with corresponding unusual high productivity, may be used as a selective property for controlling some contamination problems associated with prolonged continuous operation.

4. Domingues, L, Onnela, M-L, Teixeira, JA, Lima, N, Penttilä, M. 2000. Construction of a flocculent brewer's yeast strain secreting *Aspergillus niger* β-galactosidase. Applied Microbiology and Biotechnology **54**: 97-103.

The construction of non-flocculent and flocculent brewer's yeast strains secreting *A. niger* β-galactosidase is reported. A plasmid was constructed coding for an extracellular β-galactosidase of *Aspergillus niger* and having, as selective marker, the yeast *CUP1* gene conferring resistance to copper. The constructed strains allowed for comparison of β-galactosidase extracellular production between flocculent and non-flocculent strains with the same genetic background. The results obtained are encouraging in terms of the secretion of biological macromolecules by flocculent cells since, for the large β-galactosidase, no significant differences were found between the flocculent and non-flocculent cells. This work represents an important step towards the study of heterologous protein secretion by flocculent cells.

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1. Malfeito-Ferreira, M., Tareco, M., Loureiro, V. 1997. Fatty acid profiling: a feasible typing system to trace yeast contaminations in wine bottling plants. International J. Food Microbiol. **38**:143-155.

The long-chain fatty acid composition of yeast strains was determined for several species associated with the wine industry. The *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe*, *Brettanomyces/Dekkera* spp., *Pichia anomala*, *Pichia membranaefaciens* and *Lodderomyces elongisporus* species presented distinct fatty acid profiles after multivariate statistical analysis. The *Zygosaccharomyces rouxii* species showed profiles similar to *Z. bailii*. The use of fatty acid profiling in wine bottling plants and wines makes it possible to trace the origin of the strains responsible for spoiling the final product. In one case the origin was found at the outlet of the finishing filter and identified as *Z. bailii*. In the other case the source of contamination was discovered in the heads of the filling machine and assigned to the *P. membranaefaciens* species. The results point out the discriminating power and the industrial applicability of the technique described in this work to analyse yeast long-chain fatty acid compositions.

2. Loureiro, V., Querol, A. 1999. The prevalence and control of spoilage yeasts in foods and beverages. Trends Food Sci. Technol. **10:11**, 356-365.

3. Sancho, T., Giménez-Jurado, G., Malfeito-Ferreira, M., Loureiro, V. 2000. Zymological indicators: a new concept applied to the detection of potential spoilage yeast species associated with fruit pulps and concentrates. Food Microbiol. **18, 17**: 613-624.

In a survey of the microbial quality of raw materials used in fruit juice processing, yeast counts in fruit concentrates and pulps were found to range from <1 to 2.9×10^3 CFU/g. Ascomycetous yeasts were represented by 76% of the isolates while 24% were basidiomycetes. The identification of strains isolated by the simplified identification system (SIM) revealed 19 yeast species representing 12 genera. The most frequently isolated yeasts belonged to the genera *Saccharomyces*, *Pichia*, *Cryptococcus*, *Kluyveromyces* and *Candida*. Fatty acid yeast composition allowed the separation of contaminating yeasts belonging to one of three major groups. Group

I included yeasts without linoleic (C 18:2) and linolenic (C 18:3) fatty acids such as *S. cerevisiae*. Group II comprised yeasts without C18:3 fatty acid like *Zygosaccharomyces rouxii* and *Torulaspora delbrueckii,* and group III included yeasts with C 18:2 and C18:3 acids that belong, among others, to one of the following yeast genera: *Pichia*, *Candida*, *Kluyveromyces* or *Cryptococcus*. Species-specific PCR primers were used for the rapid detection and identification of the most dangerous species affecting fruit concentrate stability. The simplified protocol used consisted of PCR-amplification of conserved tracts in the ITS region of the rDNA unit, thus enabling the detection of potentially dangerous flora such as *Zygosaccharomyces* species and *T. delbrueckii* in contaminated fruit concentrates. Results from PCR-typing were in full agreement with the fatty acid compositions of these species. The grouping of contaminant yeasts into three main groups showed that fatty acid composition may be used to differentiate yeasts according to their technological significance. Yeasts isolated in this work as being most dangerous to product stability belong to either group II (*Z. rouxii* and *T. delbrueckii*) or group I (*Saccharomyces* spp.). Group III was comprised of several species regarded as indicators of deficiencies in "good manufacturing practices". Thus, each of the groups delineated may be considered to be a zymological indicator of technological significance. The conjugation of fatty acid profiles with PCR-typing methods may be used as a rapid detection system for contaminant yeasts. The fatty acid profiles provide a preliminary identification of yeasts potentially dangerous to product stability present within 48 h of isolation. Whereas the PCR-typing method is mainly used to confirm isolate identity, when required, after the initial diagnosis has been performed, over a period of 4 h.

4. Pereira-Dias, S., Potes, M. E., Marinho, A., Malfeito-Ferreira, M., Loureiro, V. 2000. Characterisation of yeast flora isolated from an artisanal Portuguese ewes' cheese. Int. J. Food Microbiol. **60**:55-63.

The evolution of the yeast flora was studied in the body of an artisanal semi-hard ewes' cheese made from raw milk. Mean $\log_{10}$ yeast counts per gram of cheese body ranged from 2.7 to 6.4, being the higher counts observed after a ripening period of 30 days. The yeast population decreased thereafter and, at the end of curing process, reached values similar to those of the beginning. A total of 344 yeasts strains were randomly isolated from the curd and cheese body during the 60 days long ripening period. Esterase activity was common to almost all isolates (98%) while proteolysis was observed in 12% of the total yeast population. The proportion of strains with positive glucose fermentation increased from 21% in the curd to 75% at the end of the ripening period. A total of 150 isolates representative of the physiological characteristics tested were identified by the API ID 32C system showing different degrees of quality of identification. Only 15% of the strains (23 isolates) were excellently identified being assigned to the species *Candida zeylanoides*. The other isolates presented the following degrees of quality of identification: good (13% of the strains), doubtful (43%) and good in the genus (5%). A total of 35 isolates (23%) did not match any of the identities given by the system API ID 32C. The most frequent species were *Debaryomyces hansenii* (anamorph *Candida famata*) and *Candida intermedia* which distinction depended only on pseudomycelium formation. These two species represented 9% of the yeasts in the curd increasing to 86% at the end of the ripening period.

5. Malfeito-Ferreira, M., Rodrigues, N., Loureiro, V. 2000. The influence of oxygen on the "horse sweat taint" in red wines (oral presentation). Proceedings of the 7th International Enology Symposium, 1-2 June. Pavia, Italy.

The effects of temperature and oxygen concentration on the production of 4-ethylphenol were studied in the yeast *Dekkera bruxellensis* ISA 1791. The production rate of 4-ethylphenol was more dependant on yeast growth rate than on other factors so that any environmental factor (e. g. temperature) decreasing yeast growth was also able to decrease its production. Thus, oxygen in levels up to about 8 mg/l (saturation concentration) by stimulating growth were responsible for faster production of 4-ethylphenol, thereby promoting the coming up of the "horse sweat" taint. In red wines with no measurable levels of oxygen (<2% saturation) the total amount of 4-ethylphenol produced was decreased by about 80%. However, given the high conversion rate of p-coumaric acid into 4-ethylphenol by *Dekkera*/*Brettanomyces* sp., this reduction does not prevent the development of this taint in wines with more than 1 mg/l of p-coumaric acid. In addition, the presence of oxygen stimulated the production of acetic acid increasing wine volatility. In white wines, populations of *D. bruxellensis* ISA 1791 suffered exponential death resulting in complete loss of cell viability within less than 24 hours after inoculation.

6. Loureiro, V. 2000. Spoilage yeast in foods and beverages: characterisation and ecology for diagnosis and control. Congress on "Improved traditional foods for the next century". 28-29 October, Valencia, Spain. Food Res. Inter. **33**(3-4): 247-256.

Foods and drinks with high solute concentration (fruit juices and concentrates, marzipan, salted and dry-cured meats, olives and cheeses), and foods containing preservatives (wine, beverages and sauces) were selected in order to characterise the contaminant yeast flora by rapid typing techniques. These included the testing of several types of molecular methods (e. g. RFLP, DNA-fingerprinting, PCR-based techniques), analysis of long-chain fatty acids and of isoenzymes. The PCR-based detection methods enabled a faster detection of emerging specific spoilers at earlier stages of processing. Fatty acid characterisation allowed the assessment of the most frequent types of contamination yeasts and supplied the information for the definition of relevant zymological indicators. A selected group of strains was used for further studies of mechanisms underlying the resistance/tolerance of yeasts towards preservatives (weak acids) and other stress factors (temperature, high sugar and salt concentrations). This studies enabled the acquisition of data on the basic biology of yeasts used in the development of differential and selective media for *Zygosaccharomyces bailii*, *Yarrowia lypolitica*, *Kluyveromyces marxianus* and *Dekkera* sp.

7. Carreira, A., Ferreira, L., Loureiro, V. 2001. Production of brown tyrosine pigments by the yeast *Yarrowia lipolytica*. J. Appl. Microbiol. **90**:372-379.

This work was aimed to study the mechanism of production of brown pigments from tyrosine in the yeast *Yarrowia lipolytica*.Pigment formation was followed during growth in tyrosine medium and the presence of the pigment precursor in the medium was assessed by evaluating pigment formation after removing the cells at different times of incubation. It was observed that the pigment precursor accumulated outside the cells during the exponential phase of growth, but pigment formation only occurred during the stationary phase of growth and resulted from the oxidation of the precursor. Pigment formation was repressed by glucose and L-glutamine and promoted by lactic acid, L-asparagine and glycine. Spectra of ^{1}H and ^{13}C-NMR revealed that the brown pigment was derived from tyrosine and was a polymer composed of a core of aromatic residues.The results indicate that pigments result from the extracellular accumulation and auto-oxidation of an intermediate of tyrosine catabolism. Significance and Impact of the study: This is the first report on the mechanism of pigment production from tyrosine in an yeast species.

8. Rodrigues, N., Gonçalves, G., Pereira-da-Silva, S., Malfeito-Ferreira, M., Loureiro, V. 2001. Development and use of a new medium to detect yeasts of the genera *Dekkera/Brettanomyces* sp.. J. Appl. Microbiol. **90**: 588-599.

The objectives of this work were to develop a selective and/or differential medium able to recover efficiently *Dekkera/Brettanomyces* sp. from wine related environments and to determine the relation between these yeasts and the 4-ethylphenol content in a wide range of wines.The selectivity of the developed medium was provided by the addition of ethanol as single carbon source and cycloheximide. The inclusion of bromocresol green evidenced acid producing strains. The inclusion of p-coumaric acid, substrate for the production of 4-ethylphenol, enabled the differentiation by smell of *Dekkera/Brettanomyces* sp. from all other yeast species growing in the medium. The medium was used either by plating after membrane filtration or by the Most Probable Number (MPN) technique. In 29 white and 88 red wines randomly collected, these yeasts were found only in red wines at levels up to 2500 MPN ml^{-1}, but constituted less than 1% of the total microbial flora. In red wines, 84% showed detectable amounts of 4-ethylphenol up to 4430 $\mu g\ l^{-1}$ while 28% of the white wines showed detectable levels up to 403 $\mu g\ l^{-1}$. The use of the medium proposed in this work evidenced the presence of low relative populations of *Dekkera*/*Brettanomyces* sp. even in wines contaminated by fast growing yeasts and moulds. Significance and Impact of the study: Further ecological studies on *Dekkera*/*Brettanomyces* sp. should take into account the use of highly specific culture media in order to establish their true occurrence in nature.

9. Carreira, A., Ferreira, L., Loureiro, V. (2001). Brown pigments produced by *Yarrowia lipolytica* result from extracellular accumulation of homogentisic acid. Appl. Environ. Microbiol. Accepted for publication.

Yarrowia lipolytica produces brown extracellular pigments that correlate with tyrosine catabolism. During tyrosine depletion, the yeast accumulated homogentisic acid, *p*-hydroxyphenylethanol, and *p*-hydroxyphenylacetic acid in the medium. Homogentisic acid accumulated under all aeration conditions tested, but its concentration decreased as aeration decreased. With moderate aeration, equimolar concentrations of alcohol and *p*-hydroxyphenylacetic acid (1:1) were detected, but with lower aeration the alcohol concentration was twice that of the acid (2:1). *p*-Hydroxyphenylethanol and *p*-hydroxyphenylacetic acid may result from the spontaneous disproportionation of the correspondent aldehyde, *p*-hydroxyphenylaldehyde. The catabolic pathway of tyrosine in *Y. lipolytica* involves the formation of *p*-hydroxyphenylaldehyde, which is oxidized to *p*-hydroxyphenylacetic acid, and then further oxidized to homogentisic acid. Brown pigments were produced when homogentisic acid accumulated in the medium. This acid can spontaneously oxidize and polymerize leading to the formation of pyomelanins. Mn^{2+} accelerated and intensified the oxidative polymerization of homogentisic acid, and lactic acid enhanced the stimulating role of Mn^{2+}. Alkaline conditions also accelerated pigment formation. The proposed tyrosine catabolism pathway appears to be unique for yeast, and this is the first report on a yeast producing pigments involving homogentisic acid.

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1. Guillamón, J.M., Querol, A., Jiménez, M., Huerta, T. 1993. Phenetic relationships between wine yeast strains based on electrophoretic whole-cell protein patterns. Intern. J. Food Microbiol. **18**:115-125.

In the present work, a phylogenetic study based on protein electrophoretic profiles of *Saccharomyces* strains isolated from different Spanish wine regions has been carried out. Qualitative differences between the protein electropherograms were found at inter- and intraspecific level, but not between electropherograms of strains isolated atg the same ecosystem. The numerical analysis of these results allowed us to conclude that intraspecific relationships are determined by ecological factors, as well as human influences (dispersion and artificial selection). A correlation between ecological and/or geographical origin and the relationships among strains was observed.

2. Guillamón, J.M., Barrio, E., Huerta, T., Querol, A. 1994. Rapid characterization of four species of the *Saccharomyces* sensu stricto complex according to mitochondrial DNA patterns. Inter. J. System. Bacteriol. **44**:708-714.

Several strains of the four sibling species of the genus *Saccharomyces (S. bayanus, S. cerevisiae, S. paradoxus* and *S. pastorianus*) were characterized by using a rapid and simple method of restriction analysis of mitochondrial DNA. Patterns obtained with four-cutter enconucleases (such as AluI, DdeI, HinfI and RsaI)made it possible to differentiate each species. *S. cerevisiae* and *S. paradoxus* presented a greater number of large fragments *than S. pastorianus* and *S. bayanus* with all the assay enzymes. With AluI and DdeI, species-specific bands clearly permitted differentiation between *S. pastorianus* and *S. bayanus*. To test the resolution of this method, wild *Saccharomyces* strains were analyzed. The correct assignment of these strains to a known taxon by this rapid method was confirmed by means of electrophoretic karyotyping.

3. Guillamón, J.M., Barrio, E., Querol, A. 1996. Characterization of wine yeast strains of the *Saccharomyces* genus on the basis of molecular markers: relationships between genetic distance and geographic or ecological origin. System. Appl. Microbiol. **19**: 122-132.

4. Guillamón, J.M., Sánchez, I., Huerta, T. 1997. Rapid characterization of wild and collection strains of the genus *Zygosaccharomyces* according to mitochondrial DNA patterns. FEMS Microbiol. Lett. **147**: 267-272.

Several wild and collection strains of the genus *Zygosaccharomyces* were characterized by using a rapid and simple method of restriction analysis of mitochondrial DNA. Patterns obtained with three endonucleases (HaeIII, HinfI and RsaI) made it possible to differentiate each species and to identify the wild strains, isolated from the same spoiled concentrated must, as belonging to the species Z. rouxii. The HinfI restriction enzyme produced a strain-specific pattern which allowed us to recognize that the seven wild isolates belonged to only three strains.

5. Constantí, M., Poblet, M., Arola, L., Mas, A., Guillamón, J.M. 1997. Analysis of yeast populations during alcoholic fermentation of wine in a newly established winery. Am. J. Enol. Vitic. **48**: 339-344.

Wine yeast were isolated from fermenting of Garnatxa and Xarel.lo musts prepared in a newly established winery during the 1994 and 1995 vintages. Individual strains were identified by mitochondrial DNA or rRNA coding DNA restriction analysis. A commercial starter, *Saccharomyces cerevisiae*, which was used during the first year of operation of the plant, took over the fermentations (100% of the analyzed colonies belonged to this strain) in both grape musts. This strain remained in the winery and appeared in non-inoculated fermentations during the following year. Two other main indigenous *S. cerevisiae* strains, designated as *S. cerevisiae* MF02 *and S. cerevisiae* MF03, competed with the commercial starter. Analysis of non-*Saccharomyces* strains from spontaneous fermentation of Garnatxa must showed the presence of *Hanseniaspora uvarum* and *Candida stellata* at the beginning of the process, while strains of *S. cerevisiae* began to predominate after a few days and complete the fermentation.

6. Constanti, M., Reguant, C., Poblet, M., Zamora, F., Mas, A., Guillamón, J.M. 1998. Molecular analysis of yeast population dynamics: Effect of sulphur dioxide and the inoculum in must fermentation. Inter. J. Food Microbiol**. 41:** 169-175.

The effects of sulphur dioxide and a commercial starter inoculum upon yeast population dynamics have been analysed by a molecular approach. Yeast identification from fermenting Carinyena grape musts was done by RFLP's of mtDNA and rRNA-coding DNA. As expected, the use of a commercial inoculum speeded up the starting of fermentation, while SO_2 addition limited the development of non-*Saccharomyces* species. However, this effect was also observed with yeast inoculation. Further analysis of population dynamics could lead to recommend the reduction of the dosage of SO_2 by the addition of appropriate inoculum of yeasts in the must. Furthermore, the timing for inoculum addition could be modified to allow a proper contribution of non-*Saccharomyces* species. The molecular biology analysis of population dynamics could provide a tool to efficiently reduce the dosage of SO_2 and adjust the timing of inoculum addition.

7. Guillamón, J.M., Sabaté, J., Barrio, E., Cano, J., Querol, A. 1998. Rapid Identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. Archiv. Microbiol. **169**: 387-392.

In this study, we identified a total of 33 wine yeast species and strains using the restriction patterns generated from the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene. Polymerase chain reaction (PCR) products of this rDNA region showed a high lenght variation for the different species. The size of the PCR products and the restriction analyses with three restriction endonucleases (HinfI, CfoI and HaeIII) yielded a specific restriction pattrern for each species with the exception of the corresponding anamorph and teleomorph states, which presented identical patterns. This method was applied to analyze the diversity of wine yeast species during spontaneous wine fermentation.

8. Sabaté, J., Cano, J., Querol, A., Guillamón, J.M. 1998. Diversity of *Saccharomyces* strains in wine fermentations: analysis for two consecutive years. Lett. Appl. Microbiol. **26**: 452-455.

An ecological study of *Saccharomyces cerevisiae* strains in spontaneous alcoholic fermentation has been conducted in the same winery for two consecutive years (1994 and 1995). Yeast cells were identified and characterised using mitochondrial DNA restriction analysis. Although we observed a great diversity of wild strains, we detected a sequential substitution of S. cerevisiae strains during the different phases of fermentation. Furthermore, the most frequent strains were encountered in both years, and the dynamic populations were not influenced by climatic conditions. Finally, the *Rsa*I restriction enzyme produced a species-specific pattern which allowed us to identify all the isolates as *S. cerevisiae*.

9. Sabaté, J., Guillamón, J.M., Cano, J. 2000. PCR differentiation of *Saccharomyces cerevisiae* from *Saccharomyces bayanus*/*Saccharomyces pastorianus* using specific primers. FEMS Microbiol. Lett. **193**: 255-259.

The aim of the present study was to design species-specific primers capable of distinguishing between *Saccharomyces cerevisiae*, *Saccharomyces bayanus/Saccharomyces pastorianus*. The 5'-specific primers were designed from the ITS-1 region (between positions 150 and 182 from the 3'-SSU end) and the 3'-specific primers were located in the LSU gene (positions 560-590 from the 5'-end of this gene). These primers were tested with different collections and wild strains of these species and the results showed that the primers were capable of distinguishing between *S. cerevisiae* strains and *S. bayanus/S. pastorianus*. Not enough sequence differences were found between *S. bayanus* and *S. pastorianus* to design specific primers for these species using this region. This method offers an effective tool for a quick differentiation of the *Saccharomyces* strains of the most common species involved in industrial processes.

10. Torija, M.J., Rozès, N., Poblet, M., Guillamón, J.M., Mas, A. 2001. Yeast population dynamics in spontaneous fermentations: comparisons between two different wine producing areas over a period of three years. Antonie van Leeuwenhoek Inter. J. Microbiol.: in press.

Yeast ecology, biogeography and biodiversity remain to be important and interesting topics of research. The evolution of yeasts in several cellars of two Spanish wine-producing regions was analysed for three consecutive years (1996 to 1998). No yeast starter cultures had been used in these wineries and therefore provided an ideal winemaking environment to investigate the dynamics of grape-related indigenous yeast populations. Non-*Saccharomyces* yeast species were identified by RFLPs of their rDNA, while *Saccharomyces* species and strains were identified by RFLPs of their mtDNA.This study confirmed the findings of other reports that non-*Saccharomyces* species were limited to the early stages of fermentation whilst *Saccharomyces* dominated towards the end of the alcoholic fermentation. However, it showed significant differences with previous studies such as survival of non-*Saccharomyce*s species in stages with high alcohol content and a large variability of *Saccharomyces* strains (a total of 112, all of them identified as *Saccharomyces cerevisiae*) with no clear predominance of any strain during all the fermentation, probably related to the absence of killer phenotype and lack of previous inoculation with commercial strains.

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1. Larsson, S., Reimann, A., Nilvebrant, N.-O., Jönsson, L.J. 1999. Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce. Appl. Biochem. Biotechnol. **77-79**: 91-103.

This study describes different detoxification methods to improve both cell growth and ethanol production by Baker's yeast, *Saccharomyces cerevisiae*. A dilute-acid hydrolyzate of spruce was used for the all detoxification methods tested. The changes in the concentrations of fermentable sugars and three groups of inhibitory compounds - aliphatic acids, furan derivatives, and phenolic compounds - were determined and the fermentability of the detoxified hydrolyzate was assayed. The applied detoxification methods included: treatment with alkali (sodium hydroxide or calcium hydroxide); treatment with sulfite (0.1% (w/v) or 1% (w/v) at pH 5.5 or 10); evaporation of 10% or 90% of the initial volume; anion exchange (at pH 5.5 or 10); enzymatic detoxification with the phenoloxidase laccase; and detoxification with the filamentous fungus *Trichoderma reesei*. Anion exchange at pH 5.5 or 10, treatment with laccase, treatment with calcium hydroxide, and treatment with *T. reesei* were the most efficient detoxification methods. Evaporation of 10% of the initial volume and treatment with 0.1% sulfite were the least efficient detoxification methods. Treatment with laccase was the only detoxification method that specifically removed only one group of the inhibitors, namely phenolic compounds. Anion exchange at pH 10 was the most efficient method for removing all three major groups of inhibitory compounds; however, it also resulted in loss of fermentable sugars.

2. Cassland, P., Jönsson, L.J. 1999. Characterization of a gene encoding *Trametes versicolor* laccase A and improved heterologous expression in *Saccharomyces cerevisiae* by decreased cultivation temperature. Appl. Microbiol. Biotechnol. **52**: 393-400.

Laccase can be used for enzymatic detoxification of lignocellulosic hydrolysates. A *Saccharomyces cerevisiae* strain with enhanced resistance to phenolic inhibitors and thereby improved ability to ferment lignocellulosic hydrolysates would presumably be

obtained by heterologous expression of laccase. Sequencing of the cDNA for the novel laccase gene lcc2 from the lignin-degrading basidiomycete *Trametes versicolor* showed that it encodes an isoenzyme of 499 amino-acid residues preceded by a 21-residue signal peptide. By comparison with Edman degradation data, it was concluded that lcc2 encodes an isoenzyme corresponding to laccase A. The gene product of lcc2 displays 71% identity with the previously characterized *T. versicolor* lcc1 gene product. An alignment of laccase sequences revealed that the *T. versicolor* isoenzymes in general are more closely related to corresponding isoenzymes from other white-rot fungi than to the other *T. versicolor* isoenzymes. The multiplicity of laccase is thus a conserved feature of *T. versicolor* and related species of white-rot fungi. When the *T. versicolor* lcc2 cDNA was expressed in *S. cerevisiae*, the production of active enzyme was strongly dependent on the temperature. After 3 days of incubation, a 16-fold higher laccase activity was found when a positive transformant was kept at 19 degree C instead of 28 degree C. Similar experiments with *Pichia pastoris* expressing the *T. versicolor* laccase gene lcc1 also showed that the expression level was favoured considerably by lower cultivation temperature, indicating that the observation made for the *S. cerevisiae* expression system is of general significance.

3. Larsson, S., Quintana-Sainz, A., Reimann, A., Nilvebrant, N.-O., Jönsson, L.J. 2000. Influence of lignocellulose-derived aromatic compounds on oxygen-limited growth and ethanolic fermentation by *Saccharomyces cerevisiae*. Appl. Biochem. Biotechnol. **84-86**: 617-632.

Phenolic compounds released and generated during hydrolysis inhibit fermentation of lignocellulose hydrolysates to ethanol by *Saccharomyces cerevisiae.* A wide variety of aromatic compounds form from lignin, which is partially degraded during acid hydrolysis of the lignocellulosic raw material. Aromatic compounds may also form as a result of sugar degradation and are present in wood as extractives. The influence of hydroxy-methoxy-benzaldehydes, diphenols/quinones, and phenylpropane derivatives on *S. cerevisiae* cell growth and ethanol formation was assayed using a defined medium and oxygen-limited conditions. The inhibition effected by the hydroxy-methoxy-benzaldehydes was highly dependent on the positions of the substituents. A major difference in inhibition by the oxidized and reduced form of a diphenol/quinone was observed, the oxidized form being the more inhibitory. The phenylpropane derivatives were examined with respect to difference in toxicity depending on the oxidation-reduction state of the gamma-carbon, the presence and position of unsaturated bonds in the aliphatic side chain, and the number and identity of hydroxyl and methoxyl substituents. Transformations of aromatic compounds occurring during the fermentation included aldehyde reduction, quinone reduction, and double bond saturation. Aromatic alcohols were detected as products of reductions of the corresponding aldehydes, namely hydroxy-methoxy-benzaldehydes and coniferyl aldehyde. High molecular mass compounds and the corresponding diphenol were detected as products of quinone reduction. Together with coniferyl alcohol, dihydroconiferyl alcohol was identified as a major transformation product of coniferyl aldehyde.

4. Larsson, S., Cassland, P., Jönsson, L.J. 2001. Development of a *Saccharomyces cerevisiae* strain with enhanced resistance to phenolic fermentation inhibitors in lignocellulose hydrolysates by heterologous expression of laccase. Appl. Environ. Microbiol. **67**: 1163-1170.

To improve production of fuel ethanol from renewable raw materials, laccase from the white rot fungus *Trametes versicolor* was expressed under control of the PGK1 promoter in S*accharomyces cerevisiae* to increase its resistance to phenolic inhibitors in lignocellulose hydrolysates. It was found that the laccase activity could be enhanced twofold by simultaneous overexpression of the homologous t-SNARE Sso2p. The factors affecting the level of active laccase obtained, besides the cultivation temperature, included pH and aeration. Laccase-expressing and Sso2p-overexpressing *S. cerevisiae* was cultivated in the presence of coniferyl aldehyde to examine resistance to lignocellulose-derived phenolic fermentation inhibitors. The laccase-producing transformant had the ability to convert coniferyl aldehyde at a faster rate than a control transformant not expressing laccase, which enabled faster growth and ethanol formation. The laccase-producing transformant was also able to ferment a dilute acid spruce hydrolysate at a faster rate than the control transformant. A decrease in the content of low-molecular-mass aromatic compounds, accompanied by an increase in the content of high-molecular-mass compounds, was observed during fermentation with the laccase-expressing strain, illustrating that laccase was active even at the very low levels of oxygen supplied. Our results demonstrate the importance of phenolic compounds as fermentation inhibitors and the advantage of using laccase-expressing yeast strains for producing ethanol from lignocellulose.

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1. Nedovic, V.A., Durieux, A., Van Nedervelde, L., Rosseels, P., Vandegans, J., Plaisant, A.-M., Simon, J.-P. 2000. Continuous cider fermentation with co-immobilized yeast and *Leuconostoc oenos* cells, Enz. Microbial Technol. **26**(9-10): 834-839.

Ca-alginate matrix was used to co-immobilize *Saccharomyces bayanus* and *Leuconostoc oenos* in one integrated biocatalytic system in order to perform simultaneously alcoholic and malo-lactic fermentation of apple juice to produce cider, in a continuous packed bed bioreactor. The continuous process permitted much faster fermentation compared with the traditional batch process. The flavor formation was also better controlled. By adjusting the flow rate of feeding substrate through the bioreactor, i.e. its

residence time, it was possible to obtain either "soft" or "dry" cider. However, the profile of volatile compounds in the final product was modified comparatively to the batch process, especially for higher alcohols, isoamylacetate, and diacetyl. This modification is due to different physiology states of yeast in two processes. Nevertheless, the taste of cider was quite acceptable.

2. Pjanovic, R., Goosen, M.F.A., Nedovic, V., Bugarski, B. 2000. Immobilization / encapsulation of cells using electrostatic droplet generation: experiments and theory, Minerva Biotecnologica, Special Issue on Bioencapsulation, **12**(4):241-248.

The mechanism of alginate droplet formation as well as experimental parameters for producing very small polymer microbeads (i.e. less then 100 μm diameter) using an electrostatic droplet generator were investi-gated. It was found that microbead size was a function of needle diameter, charge arrangement (i.e. electrode geometry and spacing) and strength of electric field. The process of alginate droplet formation under the influence of electrostatic forces was assessed with an image analysis/video system and revealed distinct stages; After a voltage was applied the liquid meniscus at the needle tip was distorted from a spherical shape into an inverted cone-like shape. Alginate solution flowed into this cone at an increasing rate causing formation of a neck-like filament. When this filament broke away, producing small droplets, the meniscus relaxed back to a spherical shape until flow of the polymer caused the process to start again. A mathematical model of droplet formation at the electrified needle was developed from an analysis of the forces acting on a charged droplet, and agreed well with experimental results. Finally, to assess the effect of an electric field on animal cell viability, an insect cell suspension was subjected to a high voltage. There was no detectable loss in cell viability after the voltage was applied.

3. Nedovic, V.A., Obradovic, B., Leskosek-Cukalovic, I., Vunjak-Novakovic, G. 2001. Immobilized yeast bioreactor systems for brewing – recent achievements. In: FOCUS ON BIOTECHNOLOGY, Volume IV: Engineering and Manufacturing for Biotechnology, Ph. Thonart & M. Hofman, eds., Kluwer Academic Publishers, Dordrecht, ISBN 0-7923-6927-0 (in press).

Immobilized yeast technology has attracted continual attention in the brewing industry over the last 20 years. Reasons are in many advantages offered by immobilized cell systems such as faster fermentation rates, increased volumetric productivity, and possibility of continuous operation, as compared to traditional beer production based on freely suspended cells. The essential part of the brewing process is fermentation, which consists of main and secondary fermentations. Main fermentation is responsible for the formation of most flavor compounds while the secondary fermentation provides beer maturation. These are, at the same time, the most time consuming steps in the overall process of beer production. In a traditional process main fermentation requires 5 to 7 days, while the secondary fermentation takes between 2 and 3 weeks to be completed. Implementation of immobilized yeast cell technology could provide drastic reduction of fermentation time to less than 2 days for both stages. Nowadays immobilized yeast technology is well established for secondary fermentation and alcohol-free and low-alcohol beer production. In main fermentation the situation is more complex and this process is still under scrutiny on the lab or pilot levels. This paper presents an overview of current state of immobilized yeast technology in brewing with particular emphasis on gas-lift bioreactor with alginate beads as yeast carriers.

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1. Gagiano, M., Van Dyk, D., Bauer, F.F., Lambrechts, M.G., Pretorius, I.S. 1999. Divergent regulation of the evolutionary closely related promoters of the *Saccharomyces cerevisiae STA2* and *MUC1* genes. J. Bacteriol. **181**:6496-6508.

The 5' upstream regions of the *Saccharomyces cerevisiae* glucoamylase-encoding genes, *STA1-3*, and of the *MUC1*/*FLO11* gene, which is critical for pseudohyphal development, invasive growth and flocculation, are almost identical and the genes co-regulated to a large extent. Besides representing the largest yeast promoters identified to date, these regions are of particular interest from both a functional as well as evolutionary point of view. Transcription of the genes seems indeed dependent on numerous transcription factors which integrate the information of a complex network of signaling pathways, while the very limited sequence differences between them should allow to study promoter evolution on a molecular level. To investigate the transcriptional regulation, we compared the transcription levels conferred by the *STA2* and *MUC1* promoters under various growth conditions. Our data show that transcription of both genes responded similarly to most environmental signals, but also indicated significant divergence in some aspects. We identified distinct areas within the promoters that show specific responses to the activating effect of Flo8p, Msn1p (Mss10p/Fup1p/Phd2p) and Mss11p as well as to carbon catabolite repression. We also identified the *STA10* repressive effect as the absence of Flo8p, a transcriptional activator of flocculation genes in *S. cerevisiae*.

2. Gagiano, M., Van Dyk, D, Bauer, F.F., Lambrechts, M.G., Pretorius, I.S. 1999. Msn1p/Mss10p, Mss11p and Muc1p are part of a signal transduction pathway downstream of Mep2p regulating invasive growth and pseudohyphal differentiation in *Saccharomyces cerevisiae*. Mol. Microbiol. **31**:103-116.

In *S. cerevisiae*, a network of signal transduction pathways governs the switch from yeast-type growth to pseudohyphal and invasive growth that occurs in response to nutrient limitation. Important elements of this network have been identified, including nutrient signal-receptors, GTP-binding proteins, components of the pheromone-dependent MAP kinase cascade and several transcription factors. However, the structural and functional mapping of these pathways is far from being complete. Here we present data regarding three genes, *MSS10, MSS11* and *MUC1*, which form an essential part of the signal transduction network establishing invasive growth. Both *MSS10* and *MSS11* are involved in the co-regulation of starch degradation and invasive growth. Mss11p acts downstream of Mep2p, Ras2p, and Mss10p and regulates transcription of both *STA2* and *MUC1*. Regulation of *MUC1* mediates the effect of Mss10p and Mss11p on invasive growth. In addition, our results suggest that the activity of Mss10p is independent of the invasive growth MAP kinase cascade, but that Mss11p acts either downstream of, or in conjunction with, Ste12p. We also show that starch metabolism in *S. cerevisiae* is subject to regulation by components of the MAP kinase cascade.

3. Grossmann, M.K., Pretorius, I.S. 1999. Verfahren zur Identifizierung von Weinhefen und Verbesserung der Eigenschaften von *Saccharomyces cerevisiae*: eine Übersicht. Die Weinwiss. **54**:61-72.

Molecular biology tools are appropriate methods for yeast strain identification and strain improvement. Karyotyping, DNA restriction profiles of genomic, mtDNA or 2µ DNA with and without DNA probes or RAPD-PCR technique offer a closer insight in yeast population dynamics. Classical methods for strain improvement like mutation, breeding, rare-mating and protoplast fusion are presented and their use compared with genetic engineering. Construction scheme for recombinant strains and application exemples are presented as well as regulatory aspects.

4. Pretorius, I.S., Van der Westhuizen, T.J., Augustyn, O.P.H. 1999. The importance of yeast biodiversity in South African vineyards and wineries. S. Afr. J. Enol. Vitic. **20**:61-74.

The art of winemaking is as old as human civilization and the use of yeast in this complex ecological and biochemical process dates back to ancient times. Traditionally, yeasts associated with grape berries were simply allowed to ferment the sugars to ethanol, carbon dioxide and other minor, but important, metabolites. Spontaneous fermentations are still being used in *boutique* wineries that depend more on vintage variability. Various microbes found on the surface of grape skins and the indigenous microbiota associated with winery surfaces participate in these natural wine fermentations. Yeasts of the genera *Kloeckera*, *Hanseniaspora* and *Candida* predominate in the early stages, followed by several species of *Metschnikowia* and *Pichia* (including those species that were previously assigned to the genus *Hansenula)* in the middle stages when the ethanol rises to 3-4%. The latter stages of natural wine fermentations are invariably dominated by the alcohol-tolerant strains of *Saccharomyces cerevisiae.* However, other yeasts, such as species of *Brettanomyces*, *Kluyveromyces, Schizosaccharomyces*, *Torulaspora* and *Zygosaccharomyces* also may be present during the fermentation and can occur in the resultant wine. By contrast, the rule, rather than the exception, for modern wineries depending on reliable fermentation and the production of wines with predictable quality, is the use of specially selected starter cultures of *Saccharomyces*. However, the use of such cultures may not necessarily prevent the growth and metabolic activity of indigenous, winery associated strains of *S. cerevisiae* or other wild yeasts such as *Kloeckera apiculata*, *Hanseniaspora uvarum*, *Candida stellata* and *Torulaspora delbrueckii.* It is therefore clear that both spontaneous and inoculated wine fermentations are affected by the diversity of yeasts associated with the vineyard (natural habitat) and winery (man-made niche). In light of this, focused taxonomic surveys within an ecological framework are essential to preserve and exploit the hidden oenological potential of the untapped wealth of yeast biodiversity in our wine-producing regions. To achieve this, yeast taxonomists need to continue to isolate and characterize new yeast species and strains, while wine microbiologists develop improved identification techniques that differentiate more efficiently among individual strains. At the same time such biological surveys will complement strain development and the current international effort of molecular biologists to assign a biological function to the products of each of the 6000 genes identified by computer analysis of the nucleotide sequence of the 16 chromosomes of a laboratory strain of *S. cerevisiae.* Furthermore, only when we have a much better understanding of yeast biodiversity, biogeography, ecology and the interaction within yeast communities will we be able to optimally harness gene technology that will benefit both the wine producer and the consumer.

5. Schoeman, H., Vivier, M.A., Du Toit, M., Dicks, L.M.T., Pretorius, I.S.. 1999. The development of bactericidal yeast strains by expressing the *Pediococcus acidilactici* pediocin gene (*pedA*) in *Saccharomyces cerevisiae*. Yeast **15**:647-656.

The excessive use of sulphur dioxide and other chemical preservatives in wine, beer and other fermented food and beverage products to prevent the growth of unwanted microbes holds various disadvantages for the quality of the end-products and is confronted by mounting consumer resistance. The objective of this study was to investigate the feasibility of controlling spoilage bacteria during yeast-based fermentations by engineering bactericidal strains of *Saccharomyces cerevisiae*. To test this novel concept, we have succesfully expressed a bacteriocin gene in yeast. The pediocin operon of *Pediococcus acidilactici* PAC1·0 consists of four clustered genes, namely *pedA* (encoding a 62 amino acid precursor of the PA-1 pediocin), *pedB* (encoding an immunity factor), *pedC* (encoding a PA-1 transport protein) and *pedD* (encoding a protein involved in the transport and processing of PA-1). The *pedA* gene was inserted into a yeast expression/secretion cassette and introduced as a multicopy episomal plasmid into a laboratory strain (Y294) of *S. cerevisiae*. Northern blot analysis confirmed that the *pedA* structural gene in this construct ($ADH1_P$-$MF\alpha1_S$-*pedA*-$ADH1_T$, designated *PED1*), was efficiently expressed under the control of the yeast alcohol dehydrogenase I gene promoter ($ADH1_P$) and terminator ($ADH1_T$). Secretion of the *PED1*-encoded pediocin PA-1, was directed by the yeast mating pheromone α-factor's secretion signal ($MF\alpha1_S$). The presence of biologically active antimicrobial peptides produced by the yeast transformants was indicated by agar diffusion assays against sensitive indicator bacteria (e.g., *Listeria monocytogenes* B73). Protein analysis indicated the secreted heterologous peptide to be

approximately 4·6 kDa which conforms to the expected size. The heterologous peptide was present at relatively low levels in the yeast supernatant but pediocin activity was readily detected when intact yeast colonies were used in sensitive strain overlays. This study could lead to the development of bactericidal yeast strains where *S. cerevisiae* starter cultures not only conduct the fermentations in the wine, brewing and baking industries but also act as biological control agents to inhibit the growth of spoilage bacteria.

6. Van der Westhuizen, T.J., Augustyns, O.P.H., Pretorius, I.S. 1999. The value of long-chain fatty acid analysis, randomly amplified polymorphic DNA and electrophoretic karyotyping for the characterization of wine yeast strains. S. Afr. J. Enol. Vitic. **20**:3-10.

Wine yeast strains of *Saccharomyces* had previously been classified into several different species or varieties. This classification system was based mainly on sugar fermentation and assimilation patterns. Subsequently, most of these species were reclassified as *Saccharomyces cerevisiae*. The assignment of the majority of wine yeast strains to a single species does, however, not imply that all strains of *S. cerevisiae* are equally suitable for wine fermentation. These physiological strains of *S. cerevisiae* differ significantly in their fermentation performance and their ability to contribute to the final bouquet and quality of the various types of wine and distillates. Therefore, to ensure strain authenticity, security and proper strain management, it is of cardinal importance to have reliable taxonomic techniques available to identify and characterize individual strains of commercial cultures. In this study, 18 commercial wine yeast strains were characterized in order to evaluate and compare three taxonomic techniques, namely long-chain fatty acid analysis, randomly amplified polymorphic DNA (RAPD) and electrophoretic karyotyping. As a single identification technique, electrophoretic karyotyping seems to be the most useful method for routine fingerprinting of wine yeast strains. However, we propose that the combined use of these three techniques provides the most reliable means of differentiating among commercial wine yeast strains.

7. Vivier, M.A., Sollitti, P., Pretorius, I.S. 1999. Functional analysis of a multiple AUG codons in the transcripts of two coregulated yeast genes, *STA2* and *MUC1*. Mol. Gen. Genetics **261**:11-20.

A scanning ribosome will usually initiate translation as soon as it encounters the first favourable AUG codon and only 10% of eukaryotic transcripts have more complex arrangements. These relatively few complex transcripts normally have structural features such as multiple AUGs and significant secondary structures. However, the functional relevance of these features have rarely been established. We present here a study into the functionality of the multiple AUGs in the leaders of the *STA2* and *MUC1* transcripts of the budding yeast *Saccharomyces cerevisiae*. Each of these genes contains a putative upstream open reading frame, whereas *STA2* has an additional two in-frame AUG codons 5' of the major cistron. The *STA2* gene (a representative model of the polymorphic *STA1-3* gene family), encoding an extracellular glucoamylase, is evolutionary linked to and transcriptionally coregulated with the *MUC1* gene, that encodes a mucin-like protein essential for pseudohyphal growth and cell-adhesion in *S. cerevisiae*. We show that utilization of the alternative translational start-sites of *STA2* cause the glucoamylases to differ at their N-termini, resulting in differences of their localization patterns. Mutant analysis revealed the presence of a novel secretion enhancing signal that might prove to be relevant to the alternative targeting mechanism recently uncovered in *S. cerevisiae*. We show that a short upstream open reading frame present in the leaders of *STA1-3* and *MUC1* is bypassed in a process of leaky scanning.

8. Bauer FF, Pretorius IS. 2000. Yeast stress response and fermentation efficiency: how to survive the making of wine – A review. S. Afr. J. Enol. Vitic. **21**:27-51.

Fermentation predictability and wine quality are directly dependent on wine yeast attributes that assist in the rapid establishment of numerical dominance in the early phase of wine fermentation, and that determine the ability to conduct an even and efficient fermentation to obtain a desirable alcohol degree. It is therefore not surprising that the primary selection criteria applied to most wine yeast strain development programmes relate to the overall objective of achieving an efficient conversion of grape sugar to alcohol and carbon dioxide, at a controlled rate and without the development of off-flavours. Numerous factors influence the fermentation performance of wine yeast. Following a successful inoculation of grape must with an appropriate starter culture strain, the ability of a wine yeast to adapt to and cope with the hostile environment and stress conditions prevailing in grape juice fermentation are of vital importance to fermentation performance. There is a direct correlation between fermentation efficiency and stress resistance, which refers to the ability of a yeast strain to adapt efficiently to a changing environment and unfavourable growth conditions. Successful yeast cellular adaptation to changes in extracellular parameters during wine fermentation requires the timely perception (sensing) of chemical or physical environmental parameters, followed by accurate transmission of the information to the relevant compartments of the cell. Chemical parameters perceived during wine fermentation include the availability/concentration of certain nutrients (*e.g.*, fermentable sugars, assimilable nitrogen, oxygen, vitamins, minerals, ergosterol and unsaturated fatty acids) and the presence of inhibitory substances (*e.g.*, ethanol, acetic acid, fatty acids, sulfite, phenolic phytoalexins, mycotoxins, bacterial toxins and agrochemical residues). Signals of a physical nature include temperature, pH, agitation and osmotic pressure. The sensing of these environmental signals is carried out by specific receptor proteins, most of them situated on the cellular surface. Once perceived, the information is transmitted by a network of dedicated, interconnected signal transduction pathways to the relevant cellular compartments which implement the adaptive response, a process referred to as "stress response". Intensive research has focused on elucidating the molecular mechanisms involved in stress responses, which are evolutionarily well conserved. Besides furthering our understanding of the fundamental strategies for adaptation to hostile, industrial environments, and the biological resilience of *Saccharomyces cerevisiae*, the data are of key importance to the future improvement of wine yeast strains. This review describes the different types of stress experienced by wine yeast cells during their life cycles, summarises our current knowledge of some of the most important molecular processes required for the survival of the yeast cell, and highlights the potential benefits for future yeast strain development which can be derived from this research.

9. Du Toit M, Pretorius IS. 2000. Microbial spoilage and preservation of wine: using weapons for nature's own arsenal - A review. S. Afr. J. Enol. Vitic. **21**:74-96.

The winemaking process includes multiple stages at which microbial spoilage can occur, altering the quality and hygienic status of the wine and rendering it unacceptable. The major spoilage organisms include species and strains of the yeast genera *Brettanomyces*, *Candida, Hanseniaspora*, *Pichia*, *Zygosaccharomyces* etc., the lactic acid bacterial genera *Lactobacillus*, *Leuconostoc, Pediococcus*, etc. and the acetic acid bacterial genera *Acetobacter* and *Gluconobacter*. The faults caused include bitterness and off-flavours (mousiness, ester taint, phenolic, vinegary, buttery, geranium tone), and cosmetic problems such as turbidity, viscosity, sediment and film formation. These spoilage organisms can also affect the wholesomeness of wine by producing biogenic amines and precursors of ethyl carbamate. The judicious use of chemical preservatives such as sulphur dioxide (SO_2) during the winemaking process decreases the risk of microbial spoilage, but strains vary considerably in their SO_2 sensitivity. There is, moreover, mounting consumer bias against chemical preservatives, and this review focuses on the possible use of biopreservatives in complying with the consumers' demand for "clean and green" products.

10. Khan W, Van der Westhuizen TJ, Augustyn OPH, Pretorius IS. 2000. Geographic distribution and evaluation of *Saccharomyces crerevisiae* strains isolated from vineyards in the warm, inland regions of the Western Cape in South Africa. S. Afr. J. Enol. Vitic. **21**:17-31.

The aim of this study was to examine the geographic distribution of *Saccharomyces cerevisiae* strains indigenous to 19 sites in the warmer, inland regions of the Western Cape in South Africa. These strains were compared to those isolated previously from the cooler, coastal regions of the same province by subjecting both sets of organisms to the same characterisation procedures. Thirty isolates per sampling site were isolated and the *S. cerevisiae* strains subjected to the following characterisation procedures; karyotyping using pulse field gel electrophoresis (CHEF), randomly amplified polymorphic DNA by the polymerase chain reaction technique (RAPD-PCR), sugar fermentation ability, flocculation ability, stress resistance/response and extracellular enzyme activity. When considering biodiversity per sampling site CHEF karyotypes indicated the recovery of 30 *S. cerevisiae* strains. This number was reduced to 21 when comparing banding patterns over sites. Addition of RAPD-PCR data expanded the number of unique strains to 29. Subsequent consideration of sugar fermentation data indicated that one of the strains with exactly equivalent CHEF and RAPD-PCR patterns was in fact galactose positive while the other was galactose negative. These data clearly indicate that characterisation of yeast strains by application of a single technique is not a sound practice. None of the *S. cerevisiae* strains isolated in this study occurred in the coastal regions. In addition, each site sampled in this study had its own unique collection of wine yeast strains and no strain common to all sites in the study region was found. Survival mechanisms of *S. cerevisiae* are obscure. Although we found that many of the isolated strains could grow invasively/form pseudohyphae and that these abilities could therefore contribute to the organisms overwintering ability, other mechanisms must also be involved.

11. La Grange DC, Claeyssens IM, Pretorius IS, Van Zyl WH. 2000. Co-expression of the *Trichoderma reesei* xylanase 2 (*XYN2*) and the *Bacillus pumilus* β-xylosidase (*xynB*) genes in the yeast *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. **54**:195-200.

The *xynB* gene encoding the *Bacillus pumilus* β-xylosidase was expressed separately and jointly with the *Trichoderma reesei* β-xylanase (*xyn2*) gene in the yeast *Saccharomyces cerevisiae*. Both genes were placed under the transciptional control of the glucose-derepressible alcohol dehydrogenase 2 promoter ($ADH2_P$) and terminator ($ADH2_T$) to effect secretion in *S. cerevisiae*. The fusion protein was designated Xlo1. Xlo1 produced in *S. cerevisiae* exhibited low affinity for xylobiose, but eventually hydrolyzed xylobiose and xylotriose to the monomeric constituents, D-xylose. Coproduction of Xyn2 and Xlo2 by *S. cerevisiae* led to a 25% increase in the amount of reducing sugars released from birchwood xylan compared to *S. cerevisiae* producing only the Xyn2 β-xylanase. However, no D-xylose was produced from birchwood xylan, presumably due to very low Xlo1 β-xylosidase activity and its low affinity for xylobiose.

12. Lambrechts MG, Pretorius IS. 2000. Yeast and its importance to wine aroma. S. Afr. J. Enol. Vitic. **21**:97-129.

The most mysterious aspect of wine is the endless variety of flavours that stem from a complex, completely non-linear system of interactions among many hundreds of compounds. In its widest sense, wine flavour refers to the overall impression of both aroma and taste components. Aroma is usually associated with odorous, volatile compounds; the bouquet of wine refers to the more complex flavour compounds which evolve as a result of fermentation, *élevage* and ageing. With the exception of terpenes in the aromatic grape varieties and alkoxypyrazines in the herbaceous cultivars, perceived flavour is the result of absolute amounts and specific ratios of many of these interactive compounds, rather than being attributable to a single "impact" compound. Without underestimating the complexity of these interactive effects or negating the definitive role played by the accumulated secondary grape metabolites in the varietal character of wine, this review will focus mainly on the contribution of yeast fermentation to the sensorial quality of the final product. Yeast and fermentation conditions are claimed to be the most important factors influencing the flavours in wine. Both spontaneous and inoculated wine fermentations are affected by the diversity of yeasts associated with the vineyard and winery. During the primary alcoholic fermentation of sugar, the wine yeast, *Saccharomyces cerevisiae*, together with other indigenous non-*Saccharomyces* species, produce ethanol, carbon dioxide and a number of by-products. Of these yeast-derived metabolites, the alcohols, acetates and C_4-C_8 fatty acid ethyl esters are found in the highest concentration in wine. While the volatile metabolites contribute to the fermentation bouquet ubiquitous to all young wines, the production levels of these by-products are variable and yeast strain specific. Therefore, this article also highlights the importance of untapping the hidden wealth of indigenous yeast species present on grapes, and the selection and genetic development of yeast starter culture strains with improved flavour profiles. In the future, some winemakers may prefer to use mixtures of indigenous yeast species and tailored *S. cerevisiae* strains as starter cultures to reflect the biodiversity and stylistic distinctiveness of a given region. This will help winemakers to fullfil the consumer's demand for individual wines with intact local character and to ensure the survival of wine's most enthralling aspect - its endless variety.

13. Lilly M, Lambrechts MG, Pretorius IS. 2000. The effect of increased yeast alcohol acetyltransferase activity on the sensorial quality of wine and brandy. Appl. Environ. Microbiol. **66**:744-753.

The distinctive flavor of wine, brandy and other grape-derived alcoholic beverages is affected by many compounds, including esters produced during alcoholic fermentation. The characteristic fruity odors of the fermentation bouquet are primarily due to a mixture of hexyl acetate, ethyl caproate (apple-like aroma), iso-amyl acetate (banana-like aroma), ethyl caprylate (apple-like aroma) and 2-phenylethyl acetate (fruity, flowery flavor with a honey note). The objective of this study was to investigate the feasibility of improving the aroma of wine and distillates by over-expressing one of the endogenous yeast genes that controls acetate ester production during fermentation. The synthesis of acetate esters by the wine yeast *Saccharomyces cerevisiae* during fermentation is ascribed to at least three acetyltransferase activities, namely alcohol acetyltransferase (AAT), ethanol acetyltransferase (EAT) and iso-amyl alcohol acetyltransferase (IAT). To investigate the effect of increased AAT activity on the sensory quality of Chenin blanc wines and distillates from Colombar base wines, we have over-expressed the alcohol acetyltransferase gene (*ATF1*) of *S. cerevisiae*. The *ATF1* gene, located on chromosome XV, was cloned from a widely used commercial wine yeast strain of *S. cerevisiae,* VIN13, and placed under the control of the constitutive yeast phosphoglycerate kinase gene (*PGK1*) promoter and terminator. Chromoblot analysis confirmed the integration of the modified copy of *ATF1* into the genome of three commercial wine yeast strains (VIN7, VIN13 and WE228). Northern blot analysis indicated constitutive expression of *ATF1* at high levels in these yeast transformants. The levels of ethyl acetate, iso-amyl acetate and 2-phenylethyl acetate increased 3- to 10-fold, 3.8- to 12-fold and 2- to 10-fold, respectively, depending on the fermentation temperature, cultivar and yeast strain used. The concentration of ethyl caprate, ethyl caprylate and hexyl acetate only showed minor changes, whereas the acetic acid concentration decreased by more than half. These changes in the wine and distillate composition had a pronounced effect on the solvent/chemical (associated with ethyl acetate and iso-amyl acetate), herbaceous and heads-associated aroma of the final distillate and the solvent/chemical and fruity/flowery character of the Chenin blanc wines. This study establishes the concept that the over-expression of acetyltransferase genes such as *ATF1* could profoundly affect the flavor profiles of wines and distillates deficient in aroma, thereby paving the way for the production of products maintaining a fruitier character for longer periods after bottling.

14. Pretorius, I.S. 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast **16**:675-729.

Yeasts are predominant in the ancient and complex process of winemaking. In spontaneous fermentations, there is a progressive growth pattern of indigenous yeasts with the final stages invariably being dominated by the alcohol-tolerant strains of *Saccharomyces cerevisiae.* This species is universally known as the *wine yeast* and is widely preferred for initiating wine fermentations. The primary role of wine yeast is to catalyse the rapid, complete and efficient conversion of grape sugars to ethanol, carbon dioxide and other minor, but important metabolites without the development of off-flavours. However, due to the demanding nature of modern winemaking practices and sophisticated wine markets, there is an ever-growing quest for specialised wine yeast strains possessing a wide range of optimised, improved or novel oenological properties. This review highlights the wealth of untapped indigenous yeasts with oenological potential, the complexity of wine yeasts' genetic features and the genetic techniques often used in strain development. The current status of genetically improved wine yeasts and potential targets for further strain development are outlined. In light of the limited knowledge of industrial wine yeasts' complex genomes and the daunting challenges to comply with strict statutory regulations and consumer demands regarding the future use of genetically modified strains, this review cautions against unrealistic expectations over the short term. However, the staggering potential advantages of improved wine yeasts to both the winemaker and consumer in the third millennium are pointed out.

15. Rainieri, S, Pretorius, I.S. 2000. Selection and improvement of wine yeasts. Ann. Microbiol. **50**:15-31.

The selection of wine yeasts is usually carried out within the species *Saccharomyces cerevisiae*. It aims at identifying the yeast strains that, besides fermenting grape juice vigorously and producing high ethanol yield, can also positively influence the composition and the sensorial characteristics of wine. The natural availability of yeast strains possessing an ideal combination of oenological characteristics is highly improbable. Moreover, selected *S. cerevisiae* wine strains usually produce wines with a plain aromatic profile. The extension of the selection of wine yeasts to *S. cerevisiae* not growing in oenological environments or to non-*Saccharomyces* yeasts has provided strains possessing novel and interesting oenological characteristics. Nevertheless, these strains cannot be directly used as starter cultures in wine fermentations, mainly because they are not vigorous or competitive in oenological conditions. Wine strains possessing innovative oenological traits that can influence the sensorial characteristics of wine can be constructed using genetic or molecular methods. Intraspecific *S. cerevisiae* hybridisation has provided useful oenological strains. Nevertheless, the traits of oenological interest that can be exchanged or introduced using this technique are only those commonly found in the species *S. cerevisiae*. Innovative oenological traits can be introduced or exchanged by hybridising strains belonging to different species but with a sufficient genetic affinity for them to mate. Interspecific *Saccharomyces* hybrids were found to be stable, vigorous and possessing the parental oenological traits in novel and interesting combinations. Nevertheless, they are sterile; the genetic improvement cannot therefore be taken further than the first generation. Moreover, the combination of the parental traits cannot be specifically programmed and a combination of positive traits is often the result of chance. The recent development of recombinant DNA technology has overcome the limitations of traditional genetic techniques as well as broadening the potential of wine yeast improvement.

16. Vadasz, A.S., Jagganath, D.B., Pretorius, I.S., Gupthar, A.S. 2000. Electron microscopy of the K_2 killer effect of *Saccharomyces cerevisiae* T206 on mesophilic wine yeast. Antonie van Leeuwenhoek **78**(2):117-122.

A mesophilic wine yeast, *Saccharomyces cerevisiae* CSIR Y217 K^-R^- was subjected to the K_2 killer effect of *Saccharomyces cerevisiae* T206 K^+R^+ in a liquid grape medium. The lethal effect of the K_2 mycoviral toxin was confirmed by methylene blue staining. Scanning electron microscopy of cells from challenge experiments revealed rippled cell surfaces, accompanied by cracks and pores, while those unaffected by the toxin, as in the control experiments, showed a smooth surface. Transmission electron microscopy revealed that the toxin damaged the cell wall structure and pertubed cytoplasmic membranes to a limited extent.

17. Van der Westhuizen, T.J., Augustyn, O.P.H., Khan, W., Pretorius, I.S. 2000. Seasonal variation of indigenous *Saccharomyces cerevisiae* strains isolated from Western Cape vineyards in South Africa. S. Afr. J. Enol. Vitic. **21**:10-16.

There is strong support for the use of naturally-occurring *Saccharomyces cerevisiae* strains that improve the sensory quality of wines and reflect the characteristics of a given region. Contrary to popular belief, *S. cerevisiae* is found at very low numbers on healthy, undamaged grapes and is rarely isolated from intact berries. The majority of studies on the population kinetics and geographic distribution of indigenous *S. cerevisiae* strains have not adequately focused on the variation in their numbers over a longer period of time. This paper discusses the results obtained in the first phase of a comprehensive research programme aimed at assessing how the natural population dynamics of *S. cerevisiae* are affected over the long term by abiotic factors. Indigenous strains of *S. cerevisiae* were aseptically isolated from eight sites in four areas in the coastal regions of the Western Cape, South Africa, during 1995 through 1998. Thirty colonies per site were isolated and the *S. cerevisiae* strains were characterized by electrophoretic karyotyping. Strain numbers per site varied over the four-year study period. Weather conditions resulting in severe fungal infestations and heavy applications of chemical sprays dramatically reduced the numbers of *S. cerevisiae* strains recovered during 1997. A return to normal weather patterns in 1998 resulted in a gradual recovery of the indigenous population. Indications are that some of the strains isolated are widespread in the study area and may represent yeasts typical of the area. Commercial wine yeast strains were recovered in only a few instances and the likelihood that commercial yeasts will eventually replace the natural yeast microflora in vineyards seems remote.

18. Van der Westhuizen, T.J., Augustyn, O.P.H., Pretorius, I.S. 2000. Geographical distribution of indigenous *Saccharomyces cerevisiae* strains isolated from vineyards in the coastal regions of the Western Cape in South Africa. S. Afr. J. Enol. Vitic. **21**:3-9.

Notwithstanding numerous studies on the yeast biota of grapes and grape must, the origin of the primary wine yeast *Saccharomyces cerevisiae* has been rather controversial. One school of thought claims that the primary source of *S. cerevisiae* is the vineyard, whereas another believes that ecological evidence points to a strict association with artificial, man-made environments such as wineries and fermentation plants. One of the main thrusts of these kind of investigations is to understand the succession of yeasts during fermentation of wine and to determine the actual contribution of indigenous strains of *S. cerevisiae* and wild yeast species to the overall sensorial quality of the end-product, even in guided fermentations using selected *S. cerevisiae* starter cultures. There is increasing interest in the wine community for the use of indigenous strains of *S. cerevisiae* and mixed starter cultures, tailored to reflect the characteristics of a given region. Against this background, we have launched a comprehensive and long overdue, biogeographical survey systematically cataloging yeasts in different climatic zones of the 350-year-old wine-producing regions of the Western Cape. The present paper represents the first phase of this programme aimed to preserve and exploit the hidden oenological potential of the untapped yeast biodiversity in South Africa's primary grape-growing areas. Grapes were aseptically harvested from 13 sites in five areas in the coastal regions of the Western Cape. After fermentation, 30 yeast colonies per sample were isolated and examined for the presence of *S. cerevisiae*. Five sampling sites yielded no *S. cerevisiae*. CHEF-DNA analysis revealed the presence of 46 unique karyotypes in eight of the remaining sites. No dominant strain was identified and each site had its own unique collection of strains. The number of strains per site varied from two to 15. Only in four cases did one strain appear at two sites, while only one instance of a strain occurring at three sites was recorded. All sites contained killer and sensitive strains, however, killer strains did not always dominate. Commercial strains were recovered from three sites. Although commercial yeasts dominated the micro flora at two sites, it appears that fears of commercial yeasts ultimately dominating the natural microflora seem to be exaggerated.

19. Van Rensburg, P., Pretorius, I.S. 2000. Enzymes in winemaking: harnessing natural catalysts for efficient biotransformations – A review. S. Afr. J. Enol. Vitic. **21**:52-73.

Enzymes play a definitive role in the ancient and complex process of winemaking. From a scientific and technical point of view, wine can be seen as the product of enzymatic transformation of grape juice. From the pre-fermentation stage, through fermentation, post-fermentation and aging, enzymes are the major driving forces catalysing various biotransformation reactions. These biocatalysts originate not only from the grape itself but also from yeasts and other microbes (fungi and bacteria) associated with vineyards and wine cellars. Through better understanding of these enzymatic activities, winemakers have come to learn how to control the unwanted enzymes while optimising the desired activities. Today, winemakers reinforce and extend the action of these endogenous enzymes by the judicious application of an ever-increasing spectrum of commercial enzyme preparations. These enzyme preparations are applied to winemaking with the aims of improving the clarification and processing of wine, releasing varietal aromas from precursor compounds, reducing ethyl carbamate formation and lowering alcohol levels. This review article summarises the most important enzymes applied to winemaking, the nature and structure of their substrates, and the reactions catalysed by these enzymes. This paper also reviews the limitations of the endogenous enzymes derived from grapes and microbes present in must and wine, along with the effects of commercial enzyme preparations on process technology and the quality of the final product. Prospects of developing wine yeast strains expressing tailored enzymes are also highlighted.

20. Vivier, M.A., Pretorius, I.S. 2000. Genetic improvement of grapevine: tailoring grape varieties for the third millennium – A review. S. Afr. J. Enol. Vitic. **21**:5-26.

The remarkable propagative aptitude of grapevine is one of the key factors contributing to its success as a cultivated species and to the spread of the domesticated grapevine, establishing it as one of the most important fruit species worldwide. Today there are some 8 million hectares of vineyards across the world. It is therefore fitting that the successful implementation of the powerful technology of gene manipulation in grapevine is to a large extent reliant on this regenerative ability. Currently, several varieties of grapevines have been successfully

genetically transformed, largely by employing somatic embryogenesis to generate highly regenerative target material. Especially attractive in the wine industry is the possibility of improving grapevine varieties by the addition of genes that confer useful traits, such as resistances against biotic and abiotic factors and manipulation of certain metabolic functions. In principle, gene transfer technology allows for the directed manipulation of a specific trait without altering the characteristic nature of the cultivar, permitting the improvement of the traditional cultivars while maintaining their established varietal characteristics. For the most part, targeted traits currently include disease resistance and improved berry quality. The promise of this technology is threatened by worldwide resistance to genetically modified organisms, and in the wine industry by complications surrounding the property rights and naming of transgenic vines. If it is not possible to maintain the varietal name when a transgenic vine has the same properties as the original well known variety, the significant advantages of gene technology over traditional breeding programmes are to a large extent lost. If these and other complications can be overcome, the integration of this powerful technology with traditional breeding programmes, and with other initiatives such as the study of the grapevine genome, will ensure a new era in the cultivation of this ancient species.

21. Bauer, F.F., Pretorius, I.S. 2001. Pseudohyphal and invasive growth in *Saccharomyces cerevisiae*. Focus on Biotechnology – Appl. Microbiol. **2**:109-133.

Cells of the yeast *Saccharomyces cerevisiae* can undergo profound molecular, physiological and morphological modifications in response to a limited supply of essential nutrients, in particular carbon or nitrogen sources. These include a shift in transcription patterns, the modification of the cell cycle, a change in budding pattern and strongly polarised growth. Cells having undergone these modifications do not separate after cell division is completed and form chains of elongated cells called pseudohyphae or filaments. Cells growing as filaments are able to invade agar plates and other substrates, a phenomenon referred to as invasive growth. A network of signal transduction pathways governs this switch from yeast-like growth to pseudohyphal and invasive growth. Important elements of this network have been identified, including nutrient signal-receptors, GTP-binding proteins, components of the pheromone-dependent MAP kinase cascade, cAMP, and several transcription factors. In this review, we summarise our current knowledge in this rapidly progressing field. We focus particularly on the interactions between several signal transduction modules and on the different transcription factors, which are regulated by these signaling modules.

22. Swiegers, J.H., Dippenaar, N., Pretorius, I.S., Bauer, F.F. 2001. Carnitine-dependent metabolic activities in *Saccharomyces cerevisiae*: three carnitine acetyltransferases are essential in a carnitine-dependent strain. Yeast **18**: 585-595.

L-Carnitine is required for the transfer of activated acyl-groups across intracellular membranes in eukaryotic organisms. In *Saccharomyces cerevisiae*, peroxisomal membranes are impermeable to acetyl-CoA, which is produced in the peroxisome when cells are grown on fatty acids as carbon source. In a reversible reaction catalysed by carnitine acetyltransferases (CATs), activated acetyl groups are transferred to carnitine to form acetylcarnitine which can be shuttled across membranes. Here we describe a mutant selection strategy which specifically selects for mutants affected in carnitine-dependent metabolic activities. Complementation of three of these mutants resulted in the cloning of three CAT encoding genes: *CAT2*, coding for the carnitine acetyltransferase associated with the peroxisomes and the mitochondria; *YAT1*, coding for the carnitine acetyltransferase which is presumably associated with the outer mitochondrial membrane and *YER024w* (*YAT2*), which encodes a third, previously unidentified carnitine acetyltransferase. The data also show that (i) L-carnitine and all three CATs are essential for growth on non-fermentable carbon sources in a strain with a disrupted *CIT2* gene, that (ii) Yat2p contributes significantly to total CAT activity when cells are grown on ethanol, and that (iii) the carnitine-dependent transfer of activated acetyl groups plays a more important role in cellular processes than previously realised.

23. Pretorius, I.S. 2001. Gene technology in winemaking: new approaches to an ancient art. Agriculturae Conspectus Scientificus **66**: 1-20

For the last century, the availability of pure culture yeast has improved reproducibility in wine fermentations and product quality. However, there is not a single wine yeast strain that possesses an ideal combination of oenological characteristics that are optimised for the task set by today's leading winemakers. With new developments in modern winemaking there has arisen an urgent need to modify wine yeast strains in order to take full advantage of technology and to satisfy the demands of the sophisticated wine consumers. The combined use of mutagenesis, hybridisation and recombinant DNA methods have significantly increased the genetic diversity that can be introduced into *Saccharomyces cerevisiae* strains. The overall aim of the strain development programmes extends far beyond the primary role of wine yeast to catalyse the rapid and complete conversion of grape sugars into alcohol and carbon dioxide without distorting the flavour of the final product. Starter cultures of *S. cerevisiae* must now possess a range of other properties that differ with the type and style of wine to be made and the technical requirements of the winery. Our strain development programme focuses on a number of targets that are amenable to a genetic approach, including strain security and quality control, the increase of fermentation and processing efficiencies, and the enhancement of the sensorial quality and health properties of wine and other grape-based beverages. However, successful commercialisation of transgenic wine yeasts will depend on a multitude of scientific, technical, economic, marketing, safety, regulatory, legal and ethical issues. Therefore, it would be foolish to entertain unrealistic expectations over rapid commercialisation and short-term benefits. However, it will be equally unwise to deny the potential advantages of genetically improved wine yeasts to both the winemaker and consumer in the third millennium.

24. Verstrepen, K.J., Bauer, F.F., Winderickx, J., Derdelinckx, G., Pretorius, I.S., Thevelein, J.M., Delvaux, F.R. 2001. Late fermentation expression of *FLO1* in *Saccharomyces cerevisiae*. J. Am. Soc. Brew. Chem. **52**: 69-76.

A strategy to alter the flocculation properties of nonflocculent yeast in such a way that flocculation occurred toward the end of fermentation was developed. In a double cross-over event, the wild-type *FLO1* promoter of the haploid, nonflocculent *S. cerevisiae* FY23 strain was replaced by a construct consisting of the *SMRI-410* marker gene and the *HSP30* promoter. In this way, the genomic copy of the wild-type *FLOI* open reading frame was brought under transcriptional control of the HSP30 promoter. The transformants showed strong flocculation toward the end of fermentation, resulting in a distinctly clearer beer than the beer obtained with wild-type cells. The other properties of the wild-type strain were conserved. Moreover, it was shown that the transformants were extremely stable and that flocculation could be induced earlier during fermentation by a heat-shock treatment or the addition of ethanol to the medium. These results suggest that the flocculation properties of weakly flocculent brewer's yeast strains can be improved using controlled expression of the *FLO1* gene.

International Commission on Yeasts and Yeast-like Organisms

Members of the Commission participating in the 21st International Specialized Symposium on Yeasts (ISSY XXI) in Lviv, Ukraine, are kindly invited to the ICY meeting, to be held during lunch on Wednesday, 22 August, 2001.

Lex Scheffers, Chair, ICY

Forthcoming Meetings

Kluyveromyces lactis Meeting, Orsay, France, July 6-8, 2001

Schedule: The meeting will start on Friday 6th of July with an informal get-together starting at 6pm followed by a dinner around 8 pm. Those event will take place at Hotel d'Orsay (300 meters from the RER station Le Guichet on line B). It will finish either Saturday 7th evening or at the latest Sunday 8th at noon.

Coming to Le Guichet: From CDG airport, take line B direction Saint Remy les Chevreuse and stop at Le Guichet. Check on the platform lights that Le Guichet is on (Le Guichet is one of the station serving the Orsay University). The train is usually direct but you may have to change train at Chatelet-Les Halles (same track, wait for the next one). It takes about 1h30 minutes to go there. Ticket is around 150FF. From Orly, buy a ticket to Le Guichet and take the VAL. It will stop at Antony where you will have to change to RER B direction Saint Remy les Chevreuse. Check that the correct train which stops at Le Guichet is arriving (indicated on the board). It takes about 30 minutes and costs around 50FF. Those arriving by train : connect to metro station Gare du Nord or Chatelet-les Halles and take line B, same direction as above. It takes around 40 (Chatelet) to 50 (Gare du Nord) minutes. Ticket is about 30FF. In all cases buy directly the ticket for the final destination, *i.e.* Le Guichet.

Meeting: The meeting itself will be housed in my Institute (about 800 meters from the hotel) and will start Saturday morning. I am waiting for all abstracts to decide if we keep to the day or expand it to Sunday morning. Anyway it will be finished at the latest Sunday noon. A registration fee of 700FF (to be paid at the meeting in French currency) will be asked per participant. It will cover Friday dinner, Saturday lunch and dinner, and the practical organization. Most if not all of you (depending on the number) will be housed at Hotel d'Orsay. The cost is 270FF per night for a single room and 310FF for a double one (breakfast included). There are of course other possibilities in Paris (but you will have to commute for about 40 minutes each way) or in Gif. For those of you coming with a car, the latter is also a very practical possibility. The cost is slightly higher (about 300FF per room).

Abstract (deadline June 15) They should be send as attached mail in Word 97 (98 for Mac) if possible. Otherwise, please contact me. A maximum of one page (Times 12, double interline) with about 2.5cm margins. Title should be in Bold (Times 14). The name of the speaker should be underlined. The room is equipped with slide projector, overhead projectors and video projection for Powerpoint presentations.

To receive a registration form, contact:

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Yeasts of the Third Millenium
21st International Specialized Symposium on Yeasts - 21th ISSY 2001
Biochemistry, Genetics, Biotechnology and Ecology of Non-conventional Yeasts (NCY)
Tuesday, 21 August - Saturday, 25 August, 2001, Lviv, Ukraine

The symposium will be organized by Lviv Institute of Cell Biology (formerly: Lviv Division of Institute of Biochemistry) and Lviv National University (Ukraine) together with Rzeszów Pedagogical University (Poland) under auspices of the Presidium of National Academy of Sciences of Ukraine and Ministry of Education and Science of Ukraine.

List of Sessions (preliminary): Systematics and Ecology of NCY. Genome Organization and Gene Expression. Genome

Sequencing in NCY. Metabolic Regulation and Engineering . Organelles Cell Surface: Structure and Functions . Stress Response. Heterologous Gene Expression. Industrial Applications. Medically Important Yeasts. *Saccharomyces versus* Non-*Saccharomyces*: Similarities and Differences.

Leading scientists from different fields of investigation of non-conventional yeasts will be invited to present lectures. Symposium program will include 4 plenary lectures . Each session will have 6 oral presentations by invited speakers. Some of these oral presentations will be selected from submitted poster abstracts. In addition, each session will include a poster presentation. Each session will be convened by two Go-Chairpersons.

At present, the following leading scientists have agreed to speak at the meeting: J.M. Cregg (USA), F. Sherman (USA), D. Klionsky (USA), C.P. Kurtzman (USA), P. Slonimski (France), C. Gaillardin (France) , C. Gancedo (Spain) , A. Dominguez (Spain) , S. Oliver (UK) , P. Sudbery (UK) , M. Veenhuis (The Netherlands), J. Thevelein (Belgium), G. Earth (Germany), C.P. Hollenberg (Germany) , P. Rasper (Slovenia) and others . Arrangement of the scientific program of the Symposium by the members of the International Scientific Committee is in progress.

International Scientific Committee: Gerold Barth, Dresden Technical University, Germany (*Yarrowia lipolytica*) . James M. Cregg, Keek Graduate Institute, Claremont, USA (heterologous gene expression, organelles). Graham H. Fleet, University of New South Wales, Sydney, Australia (ecology) . Laura Frontali, Rome University "La Sapienza", Italy (Kluyveromyces) . Sergei G. Inge-vechtomov, St. Petersburg University, Russia (genetics) . Cornelis P. Hollenberg, Düsseldorf University, Germany (heterologous gene expression) Cletus P. Kurtzman, Center of Agricultural Research, Peoria, USA (systematics). Jesus Pla, Madrid University, Spain (*Candida albicans*). Peter Raspor, University of Ljubljana, Slovenia (yeast diversity). Andrei A. Sibirny, Institute of Cell Biology, Lviv, Ukraine (metabolic regulation). Suresh Subramani, University of California at San Diego, La Jolla, USA (organelles). Masamichi Takagi, The University of Tokyo, Japan (*Candida maltosa*). Yuri A. Trotsenko, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia (biochemistry). Marten Veenhuis, Groningen University, The Netherlands (cell biology, organelles).

Local Organizing Committee: Andrei A. Sibirny, Inst. Cell Biol., Lviv, Chairman. Mykhailo V. Gonchar, Inst. Cell Biol., Lviv, Treasurer. Daria V. Fedorovych, Inst. Cell Biol., Lviv. Stepan P. Gudz, Lviv National University. Zbigniew Kotylak, Rzeszów Pedagogical University. Aleksandr R. Kulachkovsky, Lviv National University. Valentyn S. Pidgorsky, Inst. Microbiol. Virol., Kiev. Oleh V. Stasyk, Inst. Cell Biol., Lviv. Vira M. Ubyivovk, Inst. Cell Biol., Lviv.

Secretariat: Mykela M. Maidan, Inst. Cell Biol., Lviv (Head). Yuri R. Boretsky, Inst. Cell Biol., Lviv. Andrii Y. Voronovsky, Inst. Cell Biol., Lviv. Taras Y. Nazarko, Inst. Cell Biol., Lviv Oksana M. Moroz, Lviv National University.

Venue. Conference hall of the main building of Lviv National University, Lviv, Ukraine. This building is located in the historical centre of the city. The best hotels, museums, art exhibitions, and restaurants are located nearby. Lviv (also known as: Lvov, Lwow, Lwów, Lemberg, Leopoli) is the largest scientific, cultural and industrial city in the Western Ukraine with a population of nearly 1 million located in the geographical centre of Europe. It was founded as a fort in the mid-13th century by the Duke Danylo Galitsky of Galicia and was a former principality of Kyivan Rus.

Symposium Language. English will be the working language.

Registration Fee. Tentative registration fees (including Book of Abstracts, a bag, 8 coffee breaks, 4 lunches, get-together party and concert in Lviv opera house): Regular: 350 USD; Students: 200 USD; Accompanying persons : 100 USD. Registration is limited to 250 participants.

Accompanying person programme. Lunches, get-together party, concert in Lviv opera house.

Transportation. Lviv has daily flight connections from Kiev, Moscow and Warsaw, twice weekly (Tuesday, Friday) flights from Frankfurt /Ma in (Germany) and once weekly from Toronto (Canada). During summers, Lviv also has direct flights from New York. Lviv has direct train connections with major cities of the former Soviet Union including the Baltic republics. There are also direct train connections from Warsaw, Prague, Bratislava, Budapest, Bucharest, Varna as well as several western European cities including Berlin, Vienna and Venice. Lviv also has well developed bus connections with many cities of Europe, including Eastern Europe and some Western European cities (London, Manchester, Brussels, Amsterdam, Paris, Berlin, Frankfurt and others).

Hotel Accommodations. The average price per person per night is from 50-100 USD (Grand Hotel, Dnister, George) to 2540 USD (Lviv, Independence). All these hotels are located in downt own within walking distance (5-15 min) from the Lviv National University, where the symposium will be held. Less expensive accommodation (10-15 USD per person per night) will be available for students.

Social Programme. A get-together party will be arranged on the opening day of the Conference. The symposium dinner will be held at the Palace of Arts in the centre of old city. Two excursions will be organized by Organizing Committee: a walking tour around the historical centre of Lviv and a bus tour to Olesko castle (70 km from Lviv). Additional sightseeing tours will be available on an individual basis.

Information and Correspondence:

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XXth International Conference on Yeast Genetics and Molecular Biology
Prague, Czech Republic, August 26 to 31, 2001

The conference is supported by the Federation of European Microbiological Societies, under the auspices of The Academy of Sciences of the Czech Republic, The Charles University, Prague, and The Mayor of the city of Prague.

Honorary President: P.P. Slonimski (Gif-sur-Yvette).

International Scientific Committee: J. Berman (St.Paul), M. Breitenbach (Salzburg), D.G. Drubin (Berkeley), S.D. Emr (La Jolla), A. Goffeau (Paris), M.F. Tuite (Canterbury), S.G. Oliver (Manchester), K. Struhl (Boston), H.V. Westerhoff (Amsterdam), D.H. Wolf (Stuttgart), M. Yamamoto (Tokyo).

Local Organizing Committee: J. Hasek and E. Streiblova - chairpersons; I. Janatova - secretary; J. Horak, B. Janderova, Z. Palkova, A. Pichova, K. Sigler, A. Svoboda, H. Sychrova, J. Votruba.

Main Conference Topics. The Conference will include symposia and minisymposia by leading scientists in the following subjects: The Logic of Gene Regulation. Genomics of Yeast and Man. Cytoskeleton. Controlling Yeast. Cell Cycle and Differentiation. Prion-based Mechanism of Inheritance. Membrane Receptors, Sensors and Transporters. Secretion and Organelle Biogenesis. Protein Degradation. Genetic and Environmental Conditions affecting Yeast Cell and Colony Morphology. Workshops and poster sessions on related topics will also be covered.

Registration Fee. Regular participants: $530. Student: $300. Accompanying person: $110.

Please contact:

Secretariat YEAST 2001
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On-line registration:
www.biomed.cas.cz/yeast2001

22nd International Specialised Symposium on Yeasts
Yeast Fermentations and other Yeast Bioprocesses
(Organised under the auspices of the International Commission for Yeasts)
Pilanesberg National Park, South Africa. 25-28 March 2002

Invitation. The organising committee of the 22nd ISSY extends a cordial invitation to scientists interested in yeast fermentations and other yeast bioprocesses to participate in this specialised symposium. The theme of this symposium, which will take place in a unique setting with excellent conference amenities that will foster interactions between delegates, will be the biotechnological applications of yeasts. This will be the first time that an ISSY is held in Africa

Venue. The symposium will be hosted at the Kwa Maritane ("place of the rock") Game Lodge in the Pilanesberg National Park (www. parks-nw.co.za/pilanesberg/), 175 km (2 hours by car) northwest of Johannesburg. This venue was chosen because of its proximity to Johannesburg International airport, while also offering luxurious accommodation and excellent conference facilities, all within a 55 000 hectare wildlife paradise. A luxury coach will transport delegates early morning on 25th March from Johannesburg to Kwa Maritane and back again on the afternoon of 28th March.

Excursions. During the symposium delegates will enjoy a late afternoon game drive where lion, leopard, rhino, elephant, buffalo and many other wild animals may be encountered, as well as any of 360 bird species. For those who prefer a different type of recreation, the entertainment complex of Sun City/Lost City (http://www.suncity.co.za) is a mere five minutes away by shuttle bus.

Delegates arriving in South Africa on 24th March will spend the night in one of the recommended Johannesburg hotels. You may consider a day outing to Gold Reef City (http://www.goldreefcity.co.za/) on the outskirts of Johannesburg to experience a reconstructed mining town of the early 1900s, watch gold being poured and descend deep underground in a gold mine shaft to learn how gold is mined.

Climate. March (late summer) in this region is warm to hot with mostly clear skies, although late afternoon rain showers are not unusual.

Scientific programme. The scientific programme will comprise keynote lectures, invited short oral presentations, oral presentations selected from submitted abstracts and poster sessions. Provisional session topics: 1. Systematics, yeast preservation and starter cultures. 2. Alcoholic beverages and industrial alcohol. 3. Yeasts in food production. 4. Non-food products and processes. 5. Indigenous fermentations. 6. Physiology and regulation of yeast bioprocesses. 7. Metabolic engineering for improved or novel bioprocesses.

Congress of the South African Society for Microbiology. The 12th biennial congress of the South African Society for Microbiology will take place in Bloemfontein from 2-5 April 2002. (Bloemfontein is 425 km south of Johannesburg; 4.5 hours by car; also direct flights from Johannesburg International airport.) Delegates attending the 22nd ISSY may consider attending this national congress, which will have a number of speakers from abroad. Details will be provided with the second announcement of the 22nd ISSY.

To receive the second announcement. If you are interested in attending this symposium, please (a) Send an e-mail message to marianne@proper.co.za stating (i) ISSY 22, (ii) your name and title, (iii) your e-mail address, (iv) your mailing address, and (v) also indicate if you are considering to submit an abstract and

on which topic. You also may request a second annoucement at http://www.uovs.ac.za/facilities/nat/issy22.

Important dates.

July 2001 Second Announcement & call for papers
31 October 2001 Submission of abstracts deadline
10 December 2001 Notification of authors
25 January 2002 Early registration & accommodation booking deadline
28 February 2002 Late registration & accommodation booking deadline
25-28 March 2002 22nd ISSY

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Brief News Items

Training Program at the Göteborg Yeast Centre - Current Genetics

The yeast research groups in Göteborg, Sweden, offer support for PhD students to stay in Göteborg for a training period of three to nine months. The project pursued during this stay should be a part of their PhD training. This opportunity is possible because the European Commission has awarded a Marie Curie Training Site grant to the Göteborg Yeast Centre (www.gmm.gu.se/gyc). For projects starting in 2001 we request applications until the end of January, further deadlines will be in Oct 2001, 2002 and 2003. For details on available expertise and facilities please consult our website. The eight groups have complementary expertise in genetics, molecular biology, global expression analysis, functional genomics, fermentation physiology and biochemistry. We organise a yearly Yeast Day, a yearly Functional Genomics meeting as well as the International Conference on Yeast Genetics and Molecular Biology in 2003. To order a free information brochure reply to this mail and provide your mailing address. Information on application procedures and forms can be found under www.gmm.gu.se/gyc/marie-curie.htm. Please contact me for informal requests.

I would also like to bring to your attention that Springer Publishers and I plan to revive the journal Current Genetics with a number of changes and a promotion campaign from vol 40/1 (July 2001). I encourage you to submit research articles already now to the editorial office in Göteborg (my address) and I also welcome proposals for review articles.

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Change of Address - T. Nakase

After my retirement from Japan Collection of Microorganisms, RIKEN, at the end of Mar. 2000, I have been working in Thailand. Now I am studying the yeasts living in the natural environment in Thailand. My major themes of researches are the isolation and identification of yeasts associated with insects and ballistosporous yeasts living in the phyllosphere.

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